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Distribution and population size of the European pond turtle *Emys orbicularis* in Ljubljansko barje, Slovenia

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Abstract

The exact understanding of distribution and natural history is the necessary prerequisite for conservation of the endangered European pond turtle. In this study we examined the distribution and population size of pond turtles in Ljubljansko barje, a bog of European importance (Natura 2000 site, intended to be included into the Convention on Wetlands, the 'Ramsar list'). For doing so, we used different capture and observation methods. Four hundred kilometres of drainage ditches were investigated by foot, fish traps were set at 507 localities, and 171 pond turtles were captured and measured. We conclude that pond turtles are distributed all over Ljubljansko barje, but concentrated in just 18 km^2 (areas of Gmajnice and Draga pri Igu) out of the 170 km^2 total size of Ljubljansko barje. In these two areas reproduction was confirmed. The relative density for Gmajnice was calculated to 16 turtles per square kilometre, which is lower than in Draga pri Igu, with a minimum of 57 turtles per square kilometre. The main threats for the survival of pond turtles are habitat loss, nest destruction by agriculture, an increase of floods during the past 100 years, inappropriate cleaning out of drainage ditches, and the presence of invasive non-native turtles. To make conservation efforts for the European pond turtle more effective in Ljubljansko barje, we recommend using wire meshes for protecting nests and shifting the cleaning out of ditches to the active period of pond turtles.

Key words

Conservation, Emydidae, Natura 2000, Reptilia, threats.

Introduction

The natural history of the widely distributed European pond turtle (*Emys orbicularis*) is well-studied, especially in the northern parts of its distribution range. However, less information is available for more southern regions, in particular for the Balkan Peninsula (Fritz 2003; Fritz & Chiari 2013), where habitats are less impacted by human disturbance. Thus, studies in the Balkan Peninsula foster a better understanding of the pristine habitat requirements of pond turtles and are valuable for other more disturbed regions.

In Slovenia, located in the northwest of the Balkan Peninsula, the knowledge on the distribution and conservation status of the European pond turtle has increased in recent years (Vamberger 2009; Krofel et al. 2009; Vamberger & Kos 2011; Grželj & Grželj 2012; Vamberger et al. 2013a; Pekolj et al. 2015). Yet, until now only two detailed studies on its natural history were undertaken, one in a small part of Ljubljansko barje (Draga pri Igu) and another one in Bela krajina (Vamberger & Kos 2011; Vamberger et al. 2013a, b). Successful reproduction and healthy populations were found at both sites. Reports from the 17th century already mentioned frequent observations of European pond turtles (Valvasor 1689) for both areas. However, Sajovic (1910) claimed that in the late 19th century *E. orbicularis* became quite rare, even though Puschnig (1942) reported that, according to

Fig. 1. Ljubljansko barje, aerial view (left); Draga pri Igu, *Emys* locality (right).

Dr. Otto Wettstein, pond turtles still occur in Ljubljansko barje. Additional observations for the nearby Ljubljanica river in the city of Ljubljana were published by SAJOVIC (1914). In the last 30 years, only single observations of pond turtles were made from Ljubljansko barje (Fig. 2, green circles; TOME 1996, 2003, 2008a), except for Draga pri Igu, where a big population was found (VAMBERGER & KOS 2011). This leaves open the question of whether the pond turtle became extinct elsewhere or whether there is just a lack of information.

A major role for the decline could have played the century-long efforts to drain Ljubljansko barje (MARTINČIČ 1987), habitat fragmentation, intensified agriculture and the exploitation of pond turtles for food during the last two centuries (TOME 2008b), despite the protection of the species since 1920 (ANONYMOUS 1920). Moreover, native pond turtles could have been negatively impacted by released non-native conspecifics from the south of former Yugoslavia (Macedonia, southern Croatia) in the 1960s and 1970s (TOME 2008a, b), when several truckloads of living turtles (besides *E. orbicularis* also *Testudo hermanni*, *T. graeca*, *Mauremys rivulata*) were released in Ljubljansko barje. Additionally, in the last three decades the invasive slider turtle *Trachemys scripta*, which was traded and used as a pet, became established (VAMBERGER et al. 2012; STANDFUSS et al. 2016). In spite of all mentioned threats, it is almost a miracle that nature remained exceptionally diverse in this area (ANONYMOUS 2016b). As a consequence, Ljubljansko barje has been declared as a Natura 2000 site in 2004, to protect 25 bird species, 27 other animal species and 7 habitat types (ANONYMOUS 2004). Moreover, since 2008, the 'Landscape Park Ljubljansko barje' has been established (ANONYMOUS 2008) and currently the area is intended to be included into the Convention on Wetlands (the 'Ramsar list'; www.ljubljanskobarje.si), underlining the international value of the site. Also for *E. orbicularis*, Ljubljansko barje is of outstanding importance in Slovenia. TOME (2003) highlighted that 10–20% of the whole Slovenian pond turtle population live there. Yet, detailed investigations on the present status, individual numbers and natural history of *E. orbicularis* in Ljubljansko barje are lacking.

Our study aims at filling this gap to provide a sound local basis for conservation and to provide detailed data for a population in the northwestern Balkan Peninsula that can serve for comparisons with other parts of the distribution range. In particular, we examined the following questions: (1) How abundant is the European pond turtle in Ljubljansko barje? (2) Do we have vital populations (as indicated by age structure and regular records of hatchlings) across the whole Ljubljansko barje area and its surroundings, and (3) what are the major menaces for the species? To answer these questions, we surveyed the study area between 2008 and 2014 using different methods.

Methods

Study area

Ljubljansko barje is the largest Slovene and the southernmost European bog (MARTINČIČ 1987), a habitat type from which very few observations on the natural history of European pond turtles have been published (FRITZ 2003). The present appearance of Ljubljansko barje is a very fragmented mosaic of fields, intensively managed meadows, pastures, late-mown wet meadows, rich fen and transition mire patches, forests, tree plantations and some shrub land, all crisscrossed by an extensive network of around 6000 km of drainage ditches on 170 square kilometres (Fig. 1; TRČAK & ERJAVEC 2014). The main natural watercourses are the Ljubljanica, Iška and Želimeljščica rivers with their tributaries (LOVRENČAK & OROŽEN ADAMIČ 2001). Additional important freshwater habitats for the pond turtles are natural ponds (Podpeško jezero), larger ponds established as remainders of old clay pits (Vrhnika, Draga pri Igu, Fig. 1; BOŽIČ 2006) and small clay pit ponds in alder woods in which a

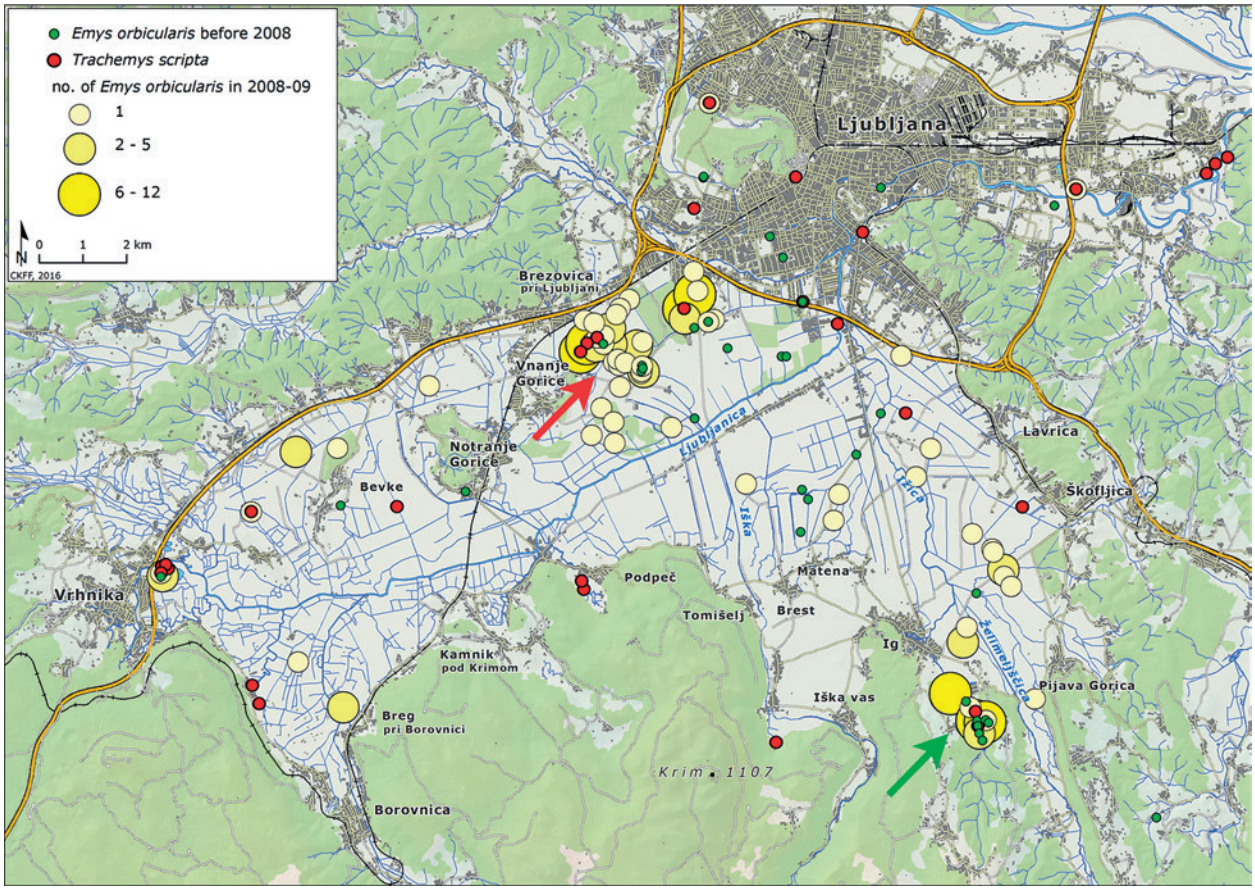

Fig. 2. Records of *Emys orbicularis* in Ljubljansko barje and surrounding areas, Slovenia. Green circles – records prior to our study (prior to 2008); yellow circles – records of present study (circle size corresponds to number of caught or observed pond turtles); red circles – records of *Trachemys scripta*. Red arrow – area of drainage ditches in Gmajnice; green arrow – Draga pri Igu ponds.

large population of *Rana arvalis* was found in last years (Stanković & Cipot 2014). Climatic conditions in the study area are mainly continental, with a mean annual temperature of 10.9°C and annual rain fall of 1352 mm (Anonymous 2016a).

Capture methods, measurements and analyses

To obtain data on distribution and population structure at Ljubljansko barje, we divided the study area into squares of 1 km^2 and examined the possible occurrence of the pond turtle in each square. For doing so, we used different capture methods. We caught pond turtles by hand or net, and by using baited fish traps in areas with deeper water (Vamberger & Kos 2011). Fieldwork was conducted only during the activity season (March to September) and only on days with favourable weather conditions. In two years we investigated 377 km of drainage ditches by foot (2008: 255 km; 2009: 121 km) and surveyed 21 km of the river Ljubljanica twice using a boat (Fig. S1). One to three kilometres of drainage ditches were investigated in most of the squares, but the invested effort depended on the quality of the habitat (Fig. S1 inset). The same was true for the fish traps which were set at 507 different localities (2008–325 localities; 2009–166 and 2014–16) and in most of the squares for 10–30 trapping days (Fig. S2). The fish traps were controlled daily. All localities of the fish traps, captured turtles and surveyed ditches were recorded using a Garmin GPS.

We measured, weighed, photographed, and individually marked turtles by marginal notching, and released them at the capture site (Vamberger & Kos 2011). We palpated adult females routinely for the presence of eggs before releasing them. Based on morphometric characters, we assigned all individuals to three different groups: male, female and subadult. We estimated the age of turtles using the average number of growth rings and shield abrasion by assigning them to five age classes (Meeske 2006; Vamberger & Kos 2011). We calculated two relative densities (number of caught turtles per 10 trapping days and number of caught turtles per 1 km of investigated drainage ditches) and the absolute number of captured turtles to compare the two different capture methods. The Peterson correlation coefficient was calculated for the number of captured turtles and survey effort (Fig. S3). Some squares were sampled repeatedly and all information was used for the statistical analysis. In Figures S1 and S2, all sampling years are summarized.

In addition, using the capture-recapture approach and the Peterson method (Krebs 1998), we estimated the

population size for pond turtles at Gmajnice and calculated the relative density per square kilometre.

To assess habitat connectivity, 'terrestrial buffer zones' of 0.5 km, 1 km and 1.5 km around the pond turtle sites were inferred using ArcGIS 9. The sizes of these buffer zones were deduced from data for individual overland movements and home ranges (FICETOLA et al. 2004; VAMBERGER & KOS 2011).

Results

The European pond turtle occurs all over Ljubljansko barje (Fig. 2, yellow circles) and was recorded from drainage ditches and ponds (single observations) with a higher individual density in the northern (Gmajnice drainage ditches; Fig. 2, red arrow) and south-eastern parts (Draga pri Igu ponds; Fig. 2, green arrow). Based on the capture-recapture approach, the relative density of pond turtles was calculated for the Gmajnice site to 16 turtles per km^2, corresponding to an estimated population size of 272 turtles (95% interval for binomial distribution: 136–850 turtles). For Draga pri Igu, the recapture rate was too low for population size estimation, but altogether 57 turtles were caught, corresponding to at least 57 turtles per km^2. Up to 5 individuals were caught in Koseški pond, an oxbow of the Ljubljanica river in Zalog and in Vrhnika. On other bigger ponds and lakes (Strahomer, Podpeč, Bistra) only the non-native pond slider *Trachemys scripta*, but no European pond turtles, were caught. Larger numbers of *T. scripta* were also present at Vrhnika, Koseze and Tivoli ponds (Fig. 2, red circles), where only low densities of *E. orbicularis* were recorded. Altogether more than 30 observations of the pond slider were made all over Ljubljansko barje (Fig. 2). In Koseze and Tivoli ponds further allochthonous turtles were found: one individual of the Florida red-bellied cooter (*Pseudemys nelsoni*) and one false map turtle (*Graptemys pseudogeographica*).

In total, 171 pond turtles were caught during the study period (113 males, 41 females, 17 subadults; data stored in the database of the Centre for Cartography of Fauna and Flora, Miklavž na Dravskem polju, Slovenia). The average carapace length for males was 135.8 mm (n=113; range: 96.88–172.12 mm), for females 157.6 mm (n=41; range: 112.96–198.45 mm) and for subadults 49.59 mm (n=17; range: 24.3–110.82 mm). The average body mass for males was 431.9 g (n=111; range 140–740 g), for females 729.7 g (n=41; range: 150–1170 g) and for subadults 116.6 g (n=8; range: 18–280 g). Among the 171 turtles 7% were juveniles below 2 years, 15% were 2–10 years old, 16% young adults, 24% adults, and 38% old adults.

Related to the invested time for capturing, most pond turtles were caught from April to June and only a few in July and August (Fig. S4). Juveniles were found in April and May and subadults until July. Only in July, the percentage of caught females was higher than for males (Fig. S5). Most of the females were caught in June and July during egg-laying in the evening. Successful reproduction was confirmed in the Draga pri Igu ponds, where 9 hatchlings and some egg-laying females were found at the banks of the Veliki pond. Gravid females were also found in Matena and Gmajnice. Subadult turtles were caught at 12 localities, but most of them at Gmajnice (Fig. S6).

If the use of terrestrial habitats around aquatic habitats is considered (up to 1.5 km; FICETOLA et al. 2004), it can be concluded that the majority of *Emys* localities is interconnected and, thus, that all pond turtles in Ljubljansko barje most likely represent one potentially panmictic population (Fig. S6).

Both capture methods (by hand or using baited fish traps) yielded good results depending on habitat or time period. The more effort was invested, the better was the catching success ($r=0.49$, $p<0.0001$; Peterson method). The highest relative densities for each method and the highest absolute numbers of captured turtles were at Gmajnice, Draga pri Igu, Vrhnika, between Ig and Črna vas and between Drenov grič and Bevke (Fig. S7).

Discussion

Distribution and abundance of *Emys orbicularis* in Ljubljansko barje

The European pond turtle was abundant all over Ljubljansko barje in VALVASOR's (1689) times, but in the 20th century the landscape changed considerably, and the turtles faced many threats (TOME 2008a, b; VAMBERGER 2009). Nevertheless, regular observations of single pond turtles have been recorded in the early 20th century from Vrhnika, Bevke, Notranje Gorice, Matena, Curnovec, Cukrarna, Vič, and Murgle, and more commonly from the Draga pri Igu ponds (SAJOVIC 1910, 1913, 1914; PUSCHNIG 1942; TOME 1996, 2003). In recent years only one thorough study was carried out at Ljubljansko barje and confirmed reproducing pond turtles in the Draga pri Igu ponds (1 km^2; VAMBERGER & KOS 2011; VAMBERGER et al. 2013a). Due to a lack of studies from Ljubljansko barje (TOME 2001, 2003), the status and exact distribution of the pond turtle across the entire area was unknown, also when Ljubljansko barje was designated as a Natura 2000 site in 2004. In this study we discovered in a 17 km^2 big area of drainage ditches in Gmajnice further reproducing pond turtles (Fig. 2, red arrow). According to our calculations, 16 turtles per km^2 could occur there, which is much less than in other European sites (FRITZ 2003) and also compared to Draga pri Igu (57 turtles/km^2). However, this estimate could be even higher if the density would be calculated per square kilometre of drainage ditches and not per total square kilometre of researched area. From all 171 caught pond turtles, more than the half was found

at Gmajnice and one third at Draga pri Igu (Fig. 2, green arrow). Besides that, only single observations were made for the remaining 152 km^2 of Ljubljansko barje (Fig. 2).

Pond turtles are known to use a 'terrestrial buffer zone' of up to 1.5 km around their aquatic habitats (Ficetola et al. 2004; Vamberger & Kos 2011). Taking this into account, it can be concluded that Ljubljansko barje most likely harbours a single interconnected population (Fig. S6). However, population density differs considerably throughout the whole area (Fig. 2), so that the habitat patches are not necessarily connected. Therefore, two more or less isolated subpopulations might exist in Draga pri Igu and Gmajnice. In any case, the new records at Koseški pond and the Ljubljanica oxbow at Zalog are of special conservation value because they are located outside of Ljubljansko barje.

Despite concerted efforts, we investigated only approximately 400 km of drainage ditches from all 6,000 km of Ljubljansko barje (Trčak & Erjavec 2014). Even though we selected potentially well-suited habitat, this means that we surveyed less than 10% of all drainage ditches. Thus, it remains unclear whether *Emys* is present or absent in the unsurveyed 90% of ditches. Using a niche modelling approach, this situation could be mitigated, but this is beyond the scope of the present paper.

Impact of the presence of non-native turtle species

We observed that the European pond turtle is rare in suitable ponds where *Trachemys scripta* was found (Fig. 2). This suggests direct competition. The fact that *T. scripta* reproduces and is becoming invasive in Ljubljansko barje (Vamberger et al. 2012; Standfuss et al. 2016) is of concern and constitutes a serious menace for *E. orbicularis*. Also other non-native turtles like *Pseudemys concinna*, *P. nelsoni*, *Pelodiscus sinensis* and *Graptemys pseudogeographica*, have recently been caught in Slovenia (Brejcha et al. 2014; Lipovšek 2013), and could pose an additional threat for pond turtles. Since the negative impact of non-native turtles, especially pond sliders (Standfuss et al. 2016), seems to be out of question, we suggest their immediate removal from all habitats.

Size and age structure

Compared to the only other well-studied Slovenian pond turtle population from Bela krajina (Vamberger et al. 2013a), the turtles in Ljubljansko barje are slightly bigger and heavier, but the differences are statistically not significant. In Bela krajina, the average carapace length for males was 123.1 mm, for females, 150.7 mm and for subadults, 49.6 mm. The average mass for males was 344 g, for females, 623 g and for subadults, 40 g (Vamberger et al. 2013a). In addition, more males were found in Ljubljansko barje (66%) compared to 50% in Bela krajina. Only 20% of the caught turtles were subadult in Ljubljansko barje compared to 50% in Bela krajina (Vamberger et al. 2013a). Most adults in Ljubljansko barje were old, suggestive of an overaged population. Both Ljubljansko barje and Bela krajina were studied during the same time period and using the same methods, so that a sampling bias can be excluded. Thus, the population in Bela krajina seems to be in a better condition than in Ljubljansko barje, but this could be due to the different habitat types in the areas.

Impact of sampling methods and sampling time

Activity periods of males, females and subadults are known to differ (Fritz 2003). Thus, for a comprehensive assessment of a local population, different sampling regimes (traps, hand-collecting, sampling time) are required (Servan 1986). In Slovenia, it is recommended to use both catching methods, at least from April to July, since these approaches are most efficient in this period (Fig. S4). Other studies have shown that after hibernation males become earlier active than females (Fritz 2003). Consequently, more males were caught during May while the best months for catching females were June and July, when the egg laying period starts (Fig. S5). It is known that some hatchlings overwinter in the nests and leave the nests only in the next spring (Fritz 2003; Vamberger 2009; Vamberger & Kos 2011) and due to that most of the juveniles were found in April and May. This observation is important as a similar phenology is expected in other regions of Central Europe and the northern Balkans, too.

Conclusions and recommendations

Except for Draga pri Igu and Gmajnice, only single pond turtles were recorded across Ljubljansko barje, suggesting that these are the last survivors in these regions. We assume that pond turtles were driven close to extinction during the 20th century, because of food consumption and habitat loss (Tome 2008a, b). Moreover, in the 1960s and 1970s, truckloads of live turtles from former Yugoslavia were released and spread over Ljubljansko barje (Tome 2008b). People from Draga pri Igu confirmed that one truckload was released exactly at Draga pri Igu. This could explain the present high numbers of pond turtles there. It is unknown whether turtles were also released at Gmajnice at that time. Future investigations using established genetic approaches for *E. orbicularis* (Lenk et al. 1999; Fritz et al. 2009; Pedall et al. 2011; Velo-Antón et al. 2011; Stuckas et al. 2014; Vamberger et al. 2015) could help to determine the genetic impact of released pond turtles and to assess whether there is still gene flow between the pond turtles from Draga pri Igu and Gmajnice.

Most of the plain area of Ljubljansko barje is intersected by agricultural land, especially by cornfields and drainage ditches. Annual floods are a natural phenomenon

there, but a surface subsidence for two metres during the past 100 years (ZAJC 2010) resulted in significantly more inundated areas than before. This is highly problematical, because many nests are destroyed. Also the intensified agriculture contributes to nest destruction and habitat loss. A further factor is the time regime of cleaning out drainage ditches. It takes place between September 30 and March 15 (ANONYMOUS 2008) and has most likely fatal consequences for pond turtles hibernating in the drainage ditches.

Thus, the long-term survival of *E. orbicularis* is threatened through habitat loss and nest destruction by intensified agriculture and floods. To mitigate the impact of floods, we recommend creating artificial nesting sites on elevated habitat structures. In addition, wire meshes should be used for covering nests, like in the Donau-Auen National Park, Austria (M. Schindler, pers. comm.), and in Brandenburg, Germany (SCHNEEWEISS & BREU 2013). In addition to a better protection against predators, the wire mesh will make nests also more visible to farmers and will therefore help avoiding incidental nest destruction. Finally, the cleaning out of ditches should be shifted to the activity period of pond turtles.

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