Research Article

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Nest Protection during a Long-term Conservation Project as a Tool to Increase the Autochthonous Population of *Emys orbicularis* (L., 1758) in Austria

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Abstract: A long-term conservation project has identified nest depredation as a major risk for the survival of the only native, reproducing population of the threatened European Pond Turtle in Austria in the Donau-Auen National Park floodplain. Thus, in 2005–2016, we utilized five designs of nest protection grids (NPGs) at the key nesting locations. Generally, NPG designs are flat 30–50 cm metal square grids with mesh size of 3 cm, attached to the ground by metal pegs. The designs, beyond their anti-predator role, had to allow mowing over them for habitat maintenance, and remain inconspicuous to visitors. NPGs were placed upon 787 nests, allowing ca. 4,500 turtles to hatch and emerge successfully. Different NPG designs provided different levels of protection, which also changed with duration of continuous use; the improvements of the NPG designs overall increased their effectiveness against native mammalian predators. However, the introduced but still rare Raccoon might be unimpeded (or undeterred). Depredation rates on protected nests did not correlate significantly with the abundance of the Red Fox (Spearman $R = -0.43$, $p = 0.22$, $n = 10$) and the European Badger ($R = 0.47$, $p = 0.17$, $n = 10$). Learning and individual experience of predators likely have stronger influence than mere abundance. We suggest that the long-term use of the NPGs has successfully allowed the population to increase.

Key words: Testudines, predation, exclusion, grid, design, method

Introduction

The present natural occurrence of the European Pond Turtle, *Emys orbicularis* (Linnaeus, 1758) in Austria is restricted to a floodplain area along a 36 km section of the Danube River, which was declared as the Donau-Auen National Park in 1997 (Gemel 2001, Schindler 2009). Due to its very localized distribution, the species was classified as Critically Endangered in the Austrian Red List (Gollmann 2007). Within a species conservation program, initiated by the National Park in 1997, nest predation was identified as a major threat (Rössler 2000). This threat is augmented by the fact that nesting sites are concentrated in a few places on an artificial flood-protection dike, which crosses the floodplain some distance from the Danube River. These conditions seem to facilitate predators in locating the nests. A minimally invasive strategy to support the population was demanded, and therefore, predator exclusion by nest protection grids was chosen, allowing natural embryonic development and dispersal of the hatchlings.

Predator-exclusion constructions are used both in marine and freshwater turtle conservation (Yerli et al. 1997, Mitrus 2000, Standing et al. 2000,

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Schneeweiss & Breu 2013). Metal grids used as predator-exclusion constructions generally do not modify the nesting environment from the natural conditions (Riley & Litzgus 2013). Because of the high level of predation, we accept the often debated possibility that predators might be attracted to the nesting sites by visual or olfactory cues we set by installing the constructions (Rollinson & Brooks 2007).

While in beach sand habitats three-dimensional cages are commonly used (with the desirable consequence of drawing people's attention), in nesting sites overgrown by vegetation mowing must be considered as a necessary measure for maintenance of the habitat. Especially on sites with high density of nests and long-term arrangements involving the use of heavy machine mowing, flat constructions are essential. In our study area, a flat design was also required so as not to attract unwanted attention by humans visiting the park. In addition, because the top of the dike is part of the international "Danube cycle path", a steady and hardly controllable stream of visitors passes directly through the nesting sites.

Nest protection grids (NPGs) are being used in the course of an ongoing long-term monitoring of the nesting sites, which started in 2005 and has operated under standardized conditions since 2007. Here, we present the results of using the NPGs in this project, aiming to provide conservation practitioners additional information for a useful tool in protecting chelonian populations from nest predators.

Materials and Methods

Study site

The Donau-Auen National Park is located along both banks of the Danube River, in eastern Austria between the capital Vienna and the border with Slovakia (48°9' N, 16°42' E; 150 m a.s.l.). Currently it covers more than 9,300 ha; of these, approx. 65% is riparian forests, 15% is meadows, and approx. 20% is covered by water.

A flood-protection dike was constructed around 1900 in a mostly West-East line on the northern river bank, with a length of ca. 36 km, transecting the floodplains. Due to the natural curves of the river bed, the dike is situated between 200 m and 1.5 km from the main river channel, reducing the regular flooding of the remaining area. At multiple locations the dike crosses side arms and oxbows, which are the main aquatic habitat for *E. orbicularis*. The trapezoidal structure rises up to 8 m above ground level; it slopes at about 30°; the base is approx. 10 m wide, while the flat top is approx. 4 m, of which 3 m are paved as part of the heavily utilized "Danube cycle path". An additional dirt road runs along the northern bottom part of the dike, providing vehicular access for official use related to maintenance of the dike and management of the National Park. The soil covering the top 50 cm of the dike consists of silt, sand and gravel in different ratios, interspersed with boulders building up the dike. Vegetation is mainly classified as Semi-dry grassland.

As part of the required maintenance of the dike in proper operating conditions, at least twice a year heavy machines are used to mow the vegetation. In the sections claimed as nesting sites, 1) mowing does not take place during the turtles' nesting and emergence periods, and 2) manually operated mowers, supervised by a researcher, are used instead of the tractors to avoid harming the clutches and the NPGs after repeated damage to the grids occurred in the beginning of the measures.

The West-East direction of the dike, its elevation and sloped sides, and the lack of tall vegetation nearby provide optimal sun exposure at its southern side. As a result of these (and other) factors, the dike seems to be one of the preferred nesting sites for this population of European Pond Turtle. However, this nesting concentration likely makes it easier for predators to find a high proportion of the nests and depredate them.

In the course of the European Pond Turtle long-term monitoring program of the National Park, four main nesting sites on the dike were identified. Nest protection activities focused on these areas, which extend to a total length of 2.15 km along the dike and cover about 86 ha.

Nest protection grid designs

Since 2005, different metal grid designs for nest protection have been used, without impacting the turtles' natural development, while providing the greatest possible protection from predators (Fig. 1). The mesh size of 3 cm was selected to allow hatchlings to emerge. In addition, all designs considered the limitations due to the specific conditions on the dike, such as mowing regulations and high number of visitors. Thus, the NPG designs were constrained, as they:

- had to be flat, so as not to impede mowing;

- had to be as small as possible, because patchy vegetation may impede flat installation due to the uneven ground surface;

- had to allow movable positions of the anchoring elements due to obstacles in the soil (stones, gravel);

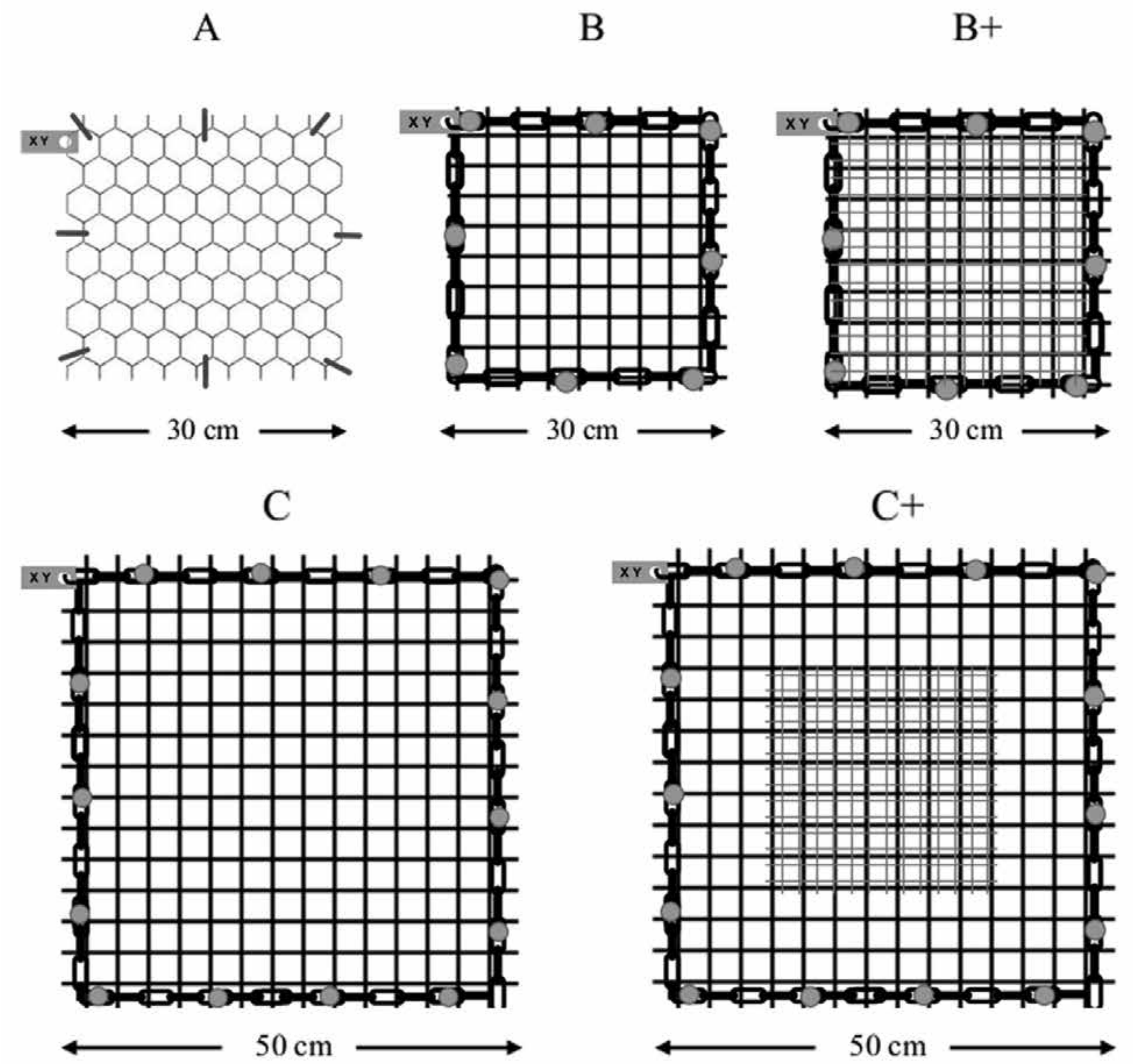

Fig. 1. Different grid designs used for predator exclusion since 2005. Anchoring elements (tent pegs, nails) are indicated as small grey lines and grey dots

- should remain inconspicuous to minimize drawing the attention of visitors;

- should be inexpensive, easy to construct, transport, install, and remove.

The NPG design was changed whenever predators adapted their behaviour in order to override the obstacle or new predators with different skills supposedly appeared in the area.

From 2005 to 2016, five different NPG designs were used. All grids were individually marked with a small aluminium plate with stamped letters. Depending on the results, the duration of use differed.

2005, Design A: The first attempts were with soft-wired flat grids with hexagonal meshes (mesh size: 3 cm, side length: 30 cm, fixed with tent pegs).

2006–2011, Design B: A new construction design consisting of spot-welded flat grids made of stainless steel was developed (square mesh size: 3 cm, side length: 30 cm). Nails (length: 16 cm) were used as anchoring elements. In order to fix the position of the nails on the grid and in spatial relation to each other, a chain was tightened around the grid. Nails were driven into the ground through the chain in a distance of 5–10 cm from one another. In order to make the construction less visible to National Park visitors, it was painted green.

2012, Design B+: In response to further predation problems, additional grids were added to design B grids and fixed to the construction with cable ties. The wires on these grids were thinner and had a mesh size of 1.5 cm. Since the small mesh size constricts hatchlings attempting to emerge through it, the additional grids had to be removed before emergence was expected.

2013, Design B+ and Design C+: On one nesting site design B+ grids were replaced with bigger-sized ones (square mesh size: 3 cm, side length: 50 cm), with additional small mesh sized grids. The material of the big grids was replaced by spot-welded iron, as it was considerably cheaper and, additionally, the rust that appears soon after installation makes painting for camouflage obsolete. The marking and anchoring system remained the same as in B+.

2014–, Design C: All grids were replaced by design C grids with the optional addition of small mesh size grid (design C+).

The cost per NPGs plus the anchoring elements varied between 4 € (design A) and 10 € (design B+), given the local prices. Weight per construction was between 0.5 kg (design A) and 1.5 kg (design C+). Preparation time for the grid designs with chains (B, B+, C, C+) is approx. 10 min per NPGs, while installation and removal in the field is max. 5 min. NPGs with additional grids (B+, C+) require a few extra minutes for preparation; removal of the additional grid before the emergence period also must be planned. To detect the NPGs (especially in tall grass) a metal detector turned out to be useful.

Data collection and analysis

Areas with high nesting activities were monitored daily between mid-May and mid-July. The earliest registered nesting date was 18th of May, the latest date – 13th of July. The late afternoon – evening period corresponds to the peak of females commencing nesting. In our experience, once egg laying is initiated, turtles are not prone to terminate it; however, prior to that they often interrupt the nesting attempt if disturbed. As the nest-protection is part of a long-term survey, in order to keep disturbance of the females by human presence at the nesting sites to a minimum, monitoring was reduced on purpose, focusing on the late evening hours (generally between 20:00–24:00 h).

Usually, one person walked back and forth slowly along the dike-slopes, alternating the relative height of their track. Since turtles could be detected for about 3 m distance using headlamps, usually three transects were needed to cover the whole side of the dike (one at the bottom, one at the middle, and one at top). We focused our search effort to the southern slope and to the upper half of the dike, since there are the highest concentrations of egg laying females.

Detected nesting females were observed with great caution. Flashlights were used to initially locate them; light intensity was reduced for closer inspection. Additionally, two trained dogs assisted to track down both females and already completed nests. In most cases, nests and females could be detected successfully in the same night; in some cases the nests were found even on the following night.

Upon the discovery of a nesting female, a small chemical glow-stick was left approx. 1 m away, to temporarily mark the location while minimizing disturbance to the turtle. Progress of nesting activity was monitored every 30–40 min. NPGs were installed immediately after the females had left the nesting site (or on the next day after a nest was found). For identification, a unique code was given to each nest. Data taken on the nest consisted of locality, coordinates and distance from the top of the dike.

Ten to twenty days after emergence was detected, nests were excavated to determine clutch size and hatching success by counting empty egg shells, undeveloped eggs and dead embryos.

Descriptive data on predation of protected nests were collected within irregular intervals: during the incubation period (July–August), during the regular controls in the emergence phase (September–October, March–April) and when empty nests were excavated. If possible, descriptive data were reclassified later into four different types:

- direct depredation (hole into the egg chamber, eggs removed);
- lateral depredation (hole dug from the edge of the grid, dug around the anchoring nails into the egg chamber);
- surface depredation (pit scratched through the grid, reaching the top of the egg chamber, with damaged eggs remain);
- depredation with removed grid (grid at least partly removed, making nest freely accessible).

The depredation rate represents the percentage of depredated nests out of the protected nests. For the analysis of a possible impact of abundance of predators on depredation rate, a Spearman Rank Correlation was calculated for 2007–2016. The years 2005 and 2006 were excluded because of the low number of protected nests. The data on annual abundance (individuals per hour of observation) of the Red Fox (*Vulpes vulpes*) and the European Badger (*Meles meles*) in the area of the whole National Park were obtained through the Österreichische Bundesforste AG (unpubl. data). Graphs and statistical analysis were performed using R 3.4.2 (R Core Team 2017) in R Studio 1.0.143 (RStudio Team 2016). Graphs were done

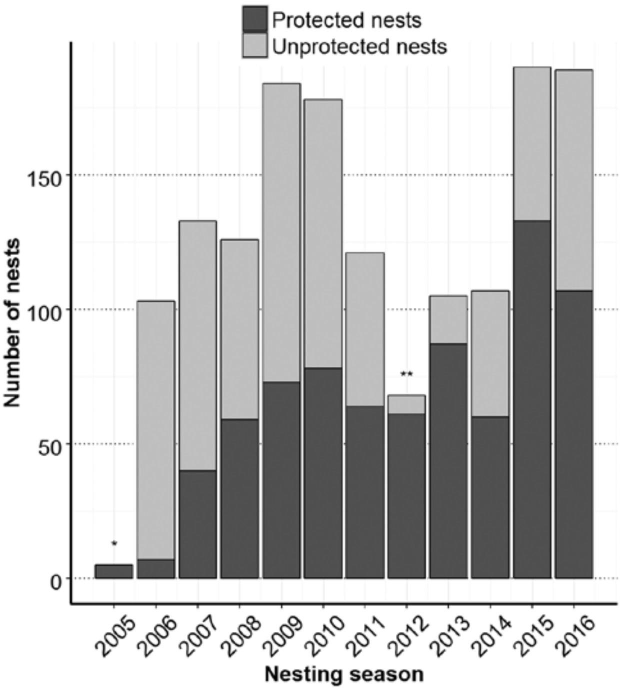

Fig. 2. Number of protected nests and (detected) unprotected nests in the study area from 2005 to 2016. Years with missing or incomplete data of unprotected nests are marked with single or double asterisk, respectively

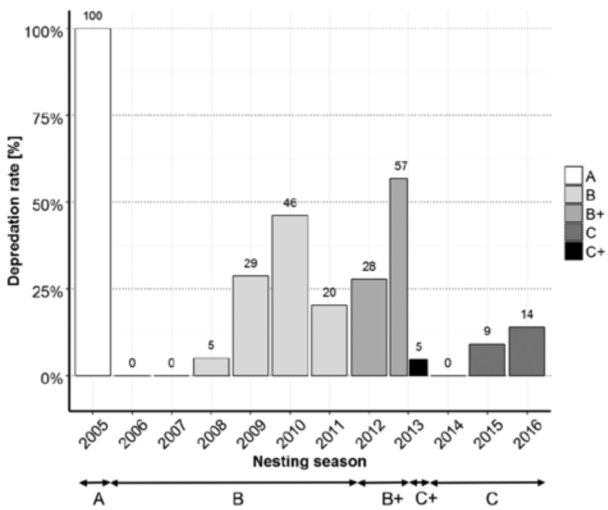

Fig. 3. Depredation rate per year and grid design. Used grid designs are presented by different colours. Additionally, duration of use of different grid designs is illustrated below the graph

with the package ggplot2 (Wickham 2009).

Results

A total of 787 nests had NPGs placed from 2005 to 2016, with annual numbers ranging from 5 (2005) to 134 (2015). Furthermore, we located 735 depredated nests without NPGs in this period (Fig.

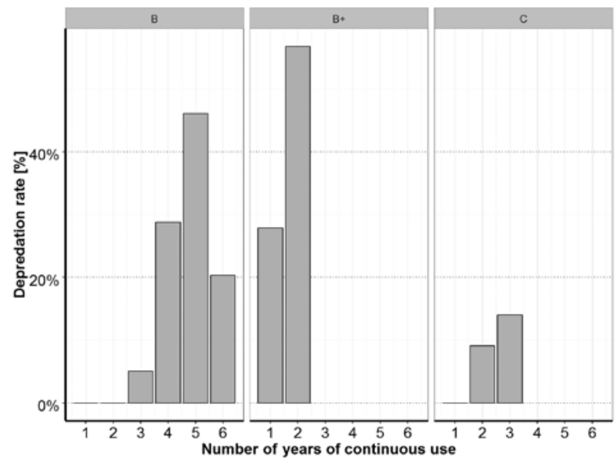

Fig. 4. Depredation rate in dependence of duration of use, for the three designs with 2+ years of continuous use (B, B+, C)

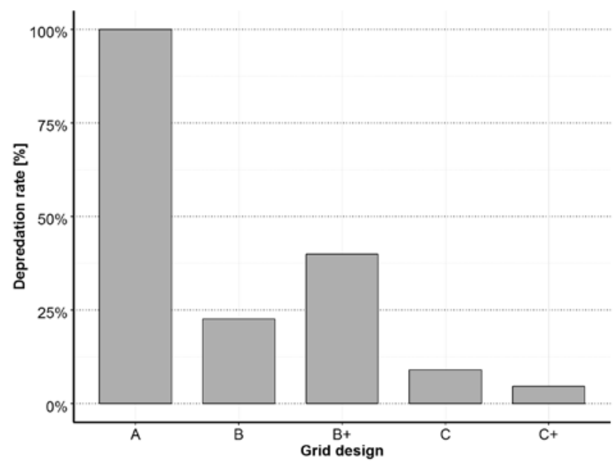

Fig. 5. Depredation rate of different grid designs over the total duration of their use

2). Approximately 4,500 hatchlings successfully emerged from protected nests.

Different NPG designs provided different levels of protection, which also changed with duration of continuous use; the improvements of the NPG designs overall increased their effectiveness (Fig. 3; but see discussion).

NPG design A was ineffective in reducing nest predation. All five nests were destroyed within the first weeks of incubation; the construction of soft wire and tent pegs was bent aside or torn out completely (depredation with removed grids). NPG design B was seemingly effective during the first two years of use (2006, 2007); however, in 2008 direct depredation occurred within 5% of the protected nests. Furthermore, in 2009 almost all protected nests were destroyed by direct depredation on the largest nesting site. In 2010 the same nesting site was periodically fenced with an electric wire as part of a sheep grazing project. While predation decreased there, in another major nesting site a large number of NPGs were torn out partly or completely (depredation with removed grids). In 2011, direct depredation increased again, like in 2009. In 2012, direct depredation during the incubation period was completely prevented by using NPG design B+, but after removing the additional small-mesh-size-grids during the hatching period, direct depredation as well as surface depredation occurred again. Furthermore, lateral depredation during the incubation period increased. In 2013 part of the NPGs were therefore replaced by design C+ grids. All types of depredation decreased at nests with C+, while most of the remaining B+ nests were laterally depredated or directly depredated after the removal of the additional grids. In 2014, when all NPGs were changed to design C, no depredation on any protected nest occurred, even though no additional grids with small mesh size were added. In 2015 and 2016 surface depredation increased, mainly during the hatching period. Since the use of NPGs with design C or C+, no nest destroyed by lateral depredation or by grid removal was documented. The few documented attempts of lateral depredation never affected the nest itself.

Further, depredation rate of different NPG designs differed depending on the duration of their use (Fig. 4). Except on the fifth year of NPG design B, there was always an increase of depredation from one year to the next, during the continuous use of the same grid design.

The depredation rate of the different NPG designs over the total duration of their use serves as a measure for their efficiency. Excluding design A (due to the small sample size), design B+ shows the highest depredation rate (40%, Fig. 5) and therefore the lowest efficiency. Design C+ is the most efficient used design with 5% depredation rate.

Correlating the depredation rates of the protected nests with the abundance of the Red Fox (Spearman $R = -0.43$, $p = 0.22$, $n = 10$) and the European Badger (Spearman $R = 0.47$, $p = 0.17$, $n = 10$) for the whole period showed no significant results.

Discussion

Our results show that the success of nest site protection with NPGs is related to its type and duration of use. Despite the very small number used of design A-grids, this type proved inefficient in protecting nests. All other grid types were quite effective in the beginning, but an increasing depredation rate was detected for consecutive years of usage for all designs in almost all cases. Only NPG design B showed no increase of depredation rate after the first year and even a decrease after the 5th year of usage. While the first could be explained by the small number of grids used in the first year, we assume that the decrease of depredation rate after the 5th year could be a consequence of the loss of single individuals of predators, likely caused by the flood of 2010. Further, the absence of depredation in 2014 could have been caused by the extremely high flood in 2013. We presume that a significant number of predators drown in these floods, decreasing the pressure they exert on the turtle nests. This is in contrast to the fact that we could not find a significant influence with the available data of the predator's abundance and depredation rate over the whole study period, as demonstrated by our results; nevertheless, an impact within single years cannot be excluded. Generally, the results emphasize that learning effects and an individual's experience have a stronger influence than the mere abundance of predators.

The assumed learning effect could also be an explanation for the low efficiency of grid design B+, as the additional grids were removed prior to the critical phase of emergence, leaving nests protected only with the less effective B-grid design. Surface depredation, which is the main reason for losses during this time, can therefore not be prevented with additional smaller sized grids. Accordingly we decided to continue with C grids (not C+), as long as direct depredation on a large scale does not occur again.

In the study area, Red Fox, European Badger and European Pine Marten (*Martes martes*) are alleged to be the main predators on turtle nests (Rössler 2000, Winterauer 2011). All of them dig by scraping away the soil, so inflexible grids should prevent any damage apart from one on the surface. However, these predators have the skills for lateral depredation as well, if the distance from the grid edge to the egg chamber is shorter than their effectively used leg-length and the lateral protection (anchoring nails) is not set densely enough. Therefore, all the native predators could have caused lateral depredation once they realized the flaws of the design. Additionally, European Badgers are probably strong enough to remove a grid by tearing it out of the soil, especially if anchoring is not done properly, e.g. due to presence of stones close to the surface.

Due to the fact that a pit of only a few centimetres is needed to reach and depredate on the uppermost eggs, we assume that surface depredation can be done by small individuals of Foxes and Martens, reaching through the NPG with their paws or by badgers with their long claws. We hypothesize that surface depredation occurs mainly in very shallow nests, due to specific substrate characteristics (such as a large amount of rocks) preventing turtles from digging a deep enough nest chamber. We have started collecting data on nest chamber measurements since 2016, but the results remain inconclusive.

The direct depredation documented in 2009–2012 likely required different skills from the predators. We assume that it was caused by a predator with groping hands that could reach through the mesh and extract the substrate bit by bit and subsequently reach the eggs. In a study, testing B-grids on various predators in different zoos, Raccoons (*Procyon lotor*) showed the most applicable results (Nagy 2011). Raccoons are not native to Austria but have been documented in the study area irregularly since the 1930s (Sackl 2001, Duscher 2016). A general increase of sightings in Austria has taken place since the 1970s with occasional evidence of reproduction since 1978 (Aubrecht 1985). Currently, a wide distribution in Austria is assumed, particularly in the lowlands and river valleys (Duscher 2016). In 2010 a Raccoon was trapped in the Donau-Auen NP (Winterauer 2011); nevertheless, neither studies in the National Park nor a master thesis on this topic could confirm the existence of Raccoons or depredation of nests by this species other than that isolated case (Stöllinger 2010, Winterauer 2011, Duscher 2016).

Flat constructions bear a higher risk of depredation, as protection is provided just by mechanical exclusion and not by a spatial extension – the distance from surface to the uppermost egg is represented by an average layer of 5 cm soil. As a consequence, mesh size must be adapted to the existing predators. The mesh size we used should prevent any damage beyond the surface, if just native species like Red Foxes, European Badgers and European Pine Martens are the expected predators. Our data on emerging hatchlings show a carapax width of max. 2.6 cm (n = 75, mean = 2.33 ± 0.15 sd, unpublished data); thus, a reduction of the mesh size to 2.5 cm (3.5 cm diagonal) or even 2 cm (2.8 cm diagonal) as tested in Hungary (B. Halpern, pers. comm.) could be considered. But these mesh sizes are not narrow enough, if Raccoons are expected predators in the area. Raccoons are skilled predators on turtle eggs and cause major damage on populations within their native range as well as in areas where they have been introduced (Schneeweiss & Breu 2013, Buzuleciu et al. 2015). A mesh size of 1 cm is needed to exclude Raccoons (Riley & Litzgus 2013, Buzuleciu et al. 2015). Grids with larger mesh size do not provide sufficient protection against this species. The same applies to narrow-meshed grids which are removed prior to emergence period. This implies that an effective protection against Raccoons through the use of NPGs eliminates the possibility for the natural emergence of hatchlings of *E. orbicularis* and most other aquatic chelonians.

Overall, the results show that predator exclusion by NPGs is a useful method to avoid major losses in turtle nests. We presume that prior to the initiation of the nest protection program the reproduction success was very low for many years, endangering the survival of the entire population. Native predators of turtle nests such as Red Foxes and European Badgers are wide-spread, essential predator species in the study area. Limited predator removal by trapping or hunting single individuals will therefore neither show the desired effect nor fit our or the National Park vision on nature conservation. However, given the number of successfully hatched turtles and a recently observed juvenile rate of more than 50% in side arms near the nesting sites (Schmidt 2017), we suggest that the long-term use of the NPGs in the NP has successfully allowed the population to increase.

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