

Theses of Doctoral (PhD) Dissertation

**EFFECTS OF CONSERVATION ACTIONS ON AMPHIBIANS IN
A LOWLAND WETLAND**

**TERMÉSZETVÉDELMI BEAVATKOZÁSOK HATÁSAI
KÉTÉLTŰEKKRE EGY SÍKVIDÉKI VIZES ÉLŐHELYEN**

Egyetemi doktori (PhD) értekezés

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A doktori értekezés betétlapja

EFFECT OF CONSERVATION MANAGEMENT ACTIONS ON AMPHIBIANS IN A LOWLAND WETLAND SYSTEM

TERMÉSZETVÉDELMI BEAVATKOZÁSOK HATÁSAI KÉTÉLTŰEKKRE EGY SÍKVIDÉKI VIZES ÉLŐHELYEN

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1. General Introduction

Amphibians are widespread ectothermic tetrapod vertebrates (Vitt & Caldwell 2014). This animal group contains 7,858 species from which 167 newly described species have been reported in 2017 (AmphibiaWeb 2018). Although amphibians have a biphasic life cycle involving both terrestrial and aquatic habitats, their reproduction is strongly tied to water (Vitt & Caldwell 2014). Ecosystems characterised by optimal precipitation and extended hydroperiods can thus maintain a diverse amphibian fauna by providing suitable habitats (Vitt & Caldwell 2014).

Many thinkers have labelled our current time as the Anthropocene in which extraordinarily rapid changes in the Earth System are caused by anthropogenic factors (Gaffney & Steffen 2017). These processes trigger the ongoing extinction of numerous animal species and mass extinction is predicted for amphibians in the close future (McCallum 2015). As of 2008, nearly half of the amphibian species were globally threatened and at least one-third of them were threatened with extinction in the wild (IUCN 2008; Stuart et al. 2008). The number of threatened and extinct species probably increased since these reports were published due to the accelerating changes in climate and spreading of amphibian infections (Hof et al. 2011; Olson et al. 2013; Alroy 2015), which have attracted considerable attention as the new driving factors of global amphibian decline triggered by human activity (Collins 2010). Moreover, population trends are still unknown for thousands of amphibian species, thus the real number of threatened and declining species can be underestimated (IUCN 2008; Bower et al. 2017).

Europe is one of the most human-altered continents, with highly fragmentated and disturbed landscapes (Sanderson et al. 2002; Ibisch et al. 2016). Habitat destruction is considered as one of the major factors of the global amphibian decline (Cushman 2006; Ohmer & Bishop 2011) which is still a significant threat in Europe besides other threats such as climate change and infections. The destruction of natural or semi-natural



habitats can be divided into three levels; fragmentation, degradation and complete loss. Due to these processes, 59% of the 85 amphibian species in Europe show a decline in their populations (Temple & Cox 2009).

Hungary is situated in the Carpathian Basin and is an integral part of the Pannonian Biogeographical Region (PBR). Because the PRB lies in the contact area of three climatic zones (Sub-mediterranean, Oceanic and Continental) and two main vegetation zones (Broadleaved Forest, Forest-steppe), it has a high biodiversity relative to the rest of Europe (Varga 2010; Vörös et al. 2015). Thus, the protection of the PRB, including endangered amphibians with the largest populations in Europe such as the Danube Crested Newt (*Triturus dobrogicus* (Kiritzescu, 1903)), is an important task for Hungary (Vörös et al. 2015).

In Hungary, amphibians are also threatened by habitat destruction (Vörös et al. 2015). According to Vörös et al. (2015) habitat loss can be characterized by 'noisy' and 'silent' phases. The noisy phase refers to the regulation of large rivers carried out mostly in the 19th century. The silent phase is the slow destruction of the aquatic and terrestrial habitats remaining after river regulations, which began in the mid 20th century (Vörös et al. 2015). Despite these processes a relatively rich amphibian fauna with 18 species is present in Hungary (Puky et al. 2005; Temple & Cox 2009).

In Hungary the first habitat protection measures started in 1939. However, only eleven wetlands including ponds, marshes and moors became protected during the next three decades (KvVM 2018). Since 1974 all amphibian species have been protected countrywide (Puky 2000). Although the first national park, the Hortobágy National Park (HNP) was established in 1973, primarily to protect the local avian fauna, this event can be considered as the second large step in the conservation of amphibians and their habitats in Hungary. The spatial expansion of protected areas including wetlands started in 1973 and the establishment of nine other national parks up to 2002 considerably increased protected areas. The third important step came with the Act LIII of 1996 on Nature



Conservation in which all alkali wetlands (soda pans) and bogs were listed as 'ex lege' protected areas. Finally, the establishment of the Natura 2000 network further increased the spatial extent of protected areas (Haraszthy 2014). Hungary currently has 10 national parks, 36 landscape protection areas, 147 nature conservation areas and 1 natural relict. In addition, 32 wetlands covering more than 200,000 hectares were listed as Wetlands of International Importance under the Ramsar Convention (Tardy 2007).

The first direct conservation action in Hungary to counter-balance the effects of habitat destruction on amphibians was carried out in 1986 to mitigate high road mortality (roadkill) rate in Common Toads (*Bufo bufo* L., 1758) caused by habitat fragmentation (Puky 1987). Since then, amphibian rescue has been organised in at least 35 localities (Vörös et al. 2015). However, only a few of the habitat reconstruction and management actions targeting amphibians have been reported, possibly due to the lack of funding. These interventions mainly focussed on species of mountaneous or hilly regions of Hungary (Vörös et al. 2015). The main cause of the degradation and loss of lowland wetlands in the 'silent' phase of habitat loss was agricultural intensification that began in the early 1950s along the drained floodplains of the Danube and Tisza rivers (Tardy 2007). The areal decline of flooded areas and wetlands limited the distribution of former populations and decreased the connectivity among them.

Conservation actions to counter-balance the impacts of habitat fragmentation, degradation and loss are thus essential in the conservation of amphibians. Scientifically sound studies can provide information on how these conservation actions should be carried out to benefit amphibian species, especially if they are studied as the imitation of natural disturbances in a large wetland and grassland system (Aradi et al. 2003; Lengyel et al. 2005).



2. Aims and Organisation of the Thesis

The aim of this PhD thesis is to fill gaps in our knowledge on the effects of several conservation interventions on lowland amphibians. Data were used from detailed surveys of the amphibian fauna and the monitoring of three conservation actions on amphibians (habitat restoration, habitat management and mitigation of amphibian road mortality) carried out in the Egyek-Pusztakócs Marsh System (EPMS, HNP, East-Hungary), a large lowland ecosystem as a model. The thesis contains four chapters, which present and discuss the results of five studies following a logical order in which each chapter follows the previous one. Because comprehensive knowledge on the status and health of the amphibian assemblages of the study site was lacking, the first task was to fill these gaps. Thus, Chapter 1 presents two studies on the amphibian fauna and on the prevalence of morphological anomalies in amphibians for the first time in the EPMS (Chapter 1 based on Henle et al. 2012; Mester et al. 2015a; Mester et al. 2017). The EPMS previously suffered from habitat destruction including three main processes (habitat loss, degradation and fragmentation) and habitats of the EPMS were later restored, managed and fenced for conservation purposes. Chapters 2-4 present the results of three studies on the effects of conservation interventions applied to counter-balance these negative processes of habitat destruction (Chapter 3 based on Mester et al. 2015b).

The aims of the four studies are detailed below. Materials and methods including the description of study sites, sampling techniques and statistical analyses are jointly described for each study according to the aims. Each chapter has its own Introduction, Results and Discussion sections. Finally, important take-home messages, including implications for practice, are given separately for each chapter. This structure allows the readers to focus on the novel results by separately follow each study along a logical order.



In Chapter 1, I addressed two questions: (i) What amphibian species with what abundance occur in the EPMS? (ii) What kind of morphological anomalies of amphibians with what total frequency occur in the EPMS?

In Chapter 2, I addressed three questions: (i) Do restoration conditions affect amphibians in restored grasslands? (ii) Do these effects differ by the age (early vs. late stages) of restoration? (iii) Do the number and abundance of amphibian species differ between restored and natural grasslands?

In Chapter 3, I addressed five questions: (i) Does management by burning and grazing influence vegetation structure, reed cover, and the diversity of the marsh? (ii) Do changes in reedbed properties benefit the anuran community? (iii) Do managements directly benefit frogs? (iv) Do burning and grazing differ in their effects on the marsh vegetation and on anurans? (v) Does the intensity of managements affect the abundance and species richness of frogs?

In Chapter 4, I addressed five questions: (i) Does the mortality of amphibians differ in space and time along a 47-km of the Main Road 33 running through Hortobágy National Park (HNP)? (ii) Does habitat composition near the road affect amphibian road mortality patterns? (iii) Does fencing as a mitigation measure reduce the mortality of amphibians? (iv) How is mortality of amphibians influenced by traffic intensity in various times of the day? (v) Was there any trend in traffic volume in the past 16 years and how did it influence estimated road mortality (roadkill) probability in various times of the day?



3. Material & Methods

3.1. Study Sites and Previous Landscape History

3.1.1. Egyek-Pusztakócs Marsh System

The studies (Chapter 1-4) were carried out in the EPMS (4,073 ha), a landscape unit in the north-eastern part of the HNP (47°34'N, 20°55'E, **Fig. 1**). The entire EPMS is included in the Natura 2000 network (both as a Special Protection Area or SPA and a Special Area for Conservation or SAC under the Birds and Habitats Directive, respectively). The EPMS is a World Heritage Site, an Important Bird and Biodiversity Area, and mentioned in the Ramsar Convention on Wetlands of International Importance. The heterogeneous landscape of the EPMS includes large alkali marshes, for example Fekete-rét, Csattag-lapos, Meggyes-lapos and other marshes (**Fig. 1**) surrounded with meadows, alkali and loess grasslands, arable lands, and native and artificial wooded areas (Aradi et al. 2003; Lengyel et al. 2005; Gőri 2007).

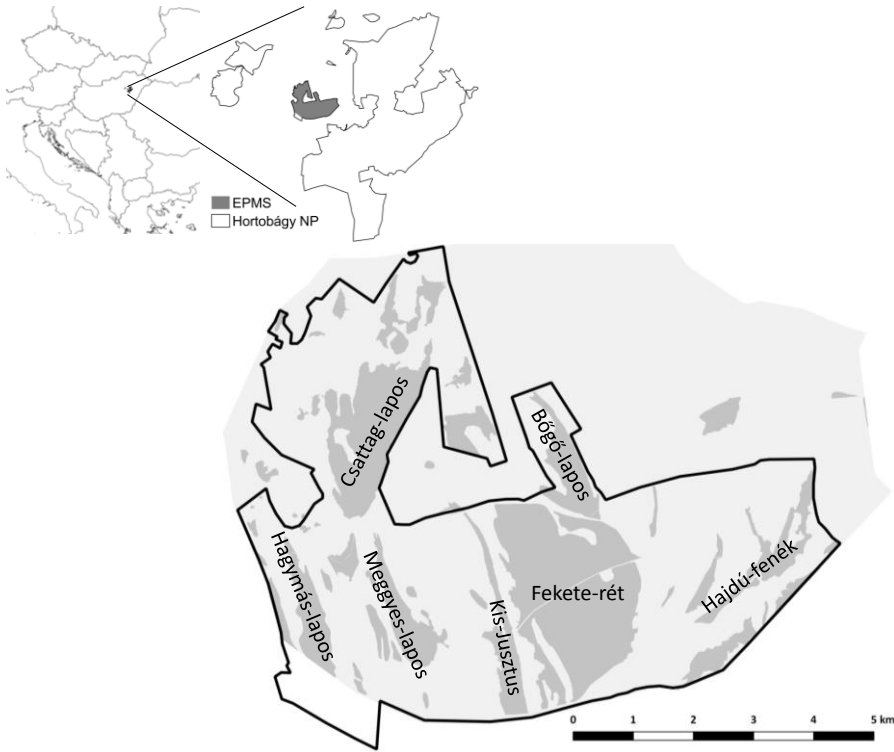


Figure 1. Geographical location of the EPMS in the national park and in E-Hungary with the name of the largest marshes.

The EPMS has been an active floodplain of the Sajó and Tisza rivers since the Pleistocene. Old floodwater beds are now lower-lying wetlands (on average 83-86 m a.s.l.) surrounded by wet meadows and separated by higher-lying and north-south oriented loess plateaus (on average 90-93 m a.s.l.). River Tisza often flooded the marshes and meadows of the EPMS from the north and they carried the river water towards the large marshes in the southern Hortobágy region. Paleoecological studies indicate that the region of Hortobágy, including the EPMS, was a fire-prone landscape in pre-human times. Later, beside wildfires, the nomad pastoralism increased the area of large grasslands (Magyari et al. 2010). Low to medium intensity extensive grazing by Hungarian Grey Cattle and sheep maintained low vegetation cover mainly throughout the Middle Ages (Török et al. 2011).



After the regulation of the River Tisza was completed in the mid-19th century, the EPMS was drained for crop production. By 1969, the proportion of arable land reached 35% of the EPMS and marshes almost completely dried out (Aradi et al. 2003; Lengyel et al. 2007). The decrease in extensive land use and increase in intensive agriculture from the mid-20th century resulted in rapid changes in vegetation cover. For instance, the proportion of reed dominated areas was 0.04% in 1892 which increased 50 times higher by 1975 with the disappearance of extensive grazing in the eastern Hortobágy region (Tóth 2003).

Changes in land use after river regulations also negatively affected the largest marshes such as Fekete-rét, a 600-ha semistatic alkali marsh (N47°33'38.60", E20°56'4.07"; 88 m a.s.l.). Charts (1855-1866) and aerial photographs (1959-1965, see **Appendix 1A**) show mosaic marsh vegetation with small open water patches, alkali shorelines, and patches of Common Reed (*Phragmites australis*) and bulrushes (*Typha* spp.). This was mainly because the marsh was regularly grazed by a number of livestock (mainly cattle) kept in numerous farms around the marsh. Later the farms were abandoned and the marsh was drained in several steps to the extent that it became completely dry in some years in the early 1970s (Aradi et al. 2003).

Parallel with agricultural intensification, linear structures such as Main Road 33 running 47 km E-W across the Hortobágy region also increased landscape and local scale fragmentation, degradation and loss of habitats. For example, this road effectively separates several marshes in the southern part of the EPMS. Before the 1950s, Main Road 33 was characterised by very low traffic, when it was linked to Main Road 3, a primary road with high traffic. Currently the daily traffic intensity is on average 2616 vehicles in the 47-km Hortobágy section. Most of this section is straight with a few slight curves. The road is a two-way paved country road with a posted 90-km/h speed limit for non-residential areas. Lanes are 3.5-m-wide and road shoulders are on average 1-m-wide. The road has a 1-m-high embankment with approximately 4-m-wide ramps on



both side covered by grass vegetation. A total of 13 drainage culverts can be found in the embankment from which at least one has been built as a crossing tunnel for amphibians (Puky 2003). The HNP directorate installed plastic fences (height: c. 1 m) on certain sections of the road in 2012 to reduce the mortality of amphibians.

3.1.2. Local Conservation Interventions

In the first large (>4000-ha) landscape-scale rehabilitation in Hungary, the hydrological supply was restored by canals (1976-1997) that brought water from the River Tisza to the alkali marshes of the EPMS, which have revitalised spectacularly. However, the constant water level, lack of grazing, and winter reed harvesting caused the spread of dense reedbeds (**Appendix 1B**).

In the second part of the landscape-scale rehabilitation, a large-scale marsh and grassland restoration programme was carried out between 2004 and 2009 with financial support from the LIFE-Nature programme (<http://life2004.hnp.hu>) (Lengyel et al. 2012). First, to restore the degraded marshes, low-intensity grazing by Hungarian Grey Cattle (2006-) and burning of old and homogeneous reed (2007, 2009) were implemented on 300 ha in Fekete-rét marsh. Second, grassland restoration was carried out by sowing alkali and loess seed mixtures on 760 ha of former cropland between 2005 and 2008 (Rácz et al. 2013; Mérő et al. 2015b).



3.2. Study Designs

3.2.1. Chapter 1

Collecting Faunistical Data

Data were collected on the occurrence of amphibian species in general faunistical surveys in the entire EPMS and in targeted surveys (detailed in Chapter 2-4). In the general surveys, three methods were used to detect amphibians between 2010 and 2016: (i) visual observations including visual encounter surveys (VES), dip netting, and road transect surveys (n = 99 locations), (ii) daylight and nocturnal call detection monitoring (n = 40 locations), (iii) bottle traps (n = 37 locations) (Dodd et al. 2012). Call detection was used to identify *Pelophylax* ‘water frogs’ (*Pelophylax* spp.) (Anthony & Puky 2001; Arnold & Ovenden 2005), because these anurans cannot be properly identified using only morphology due to their complex hybridization mechanism (Glandt 2015). However, if identification of a calling individual was considered uncertain it was registered as a *Pelophylax* spp. specimen.

In the targeted surveys, two more additional methods were used: (i) call detection and VES applied in transects in marshes (n = 49 locations) and (ii) Barber pitfall traps used in grasslands (n = 268 locations) (Heyer et al. 1994; Korsós 1997; Anthony & Puky 2001; Puky 2001a). Barber traps were originally used to survey carabid beetles and ground-dwelling spiders on former croplands (Lengyel et al. 2013). Collecting data on amphibians caught inadvertently by the traps was started in June 2010, when numerous amphibians were found in the traps, related to the extremely wet weather of the summer of 2010 (Mester et al. 2015a).



Collecting Data on Morphological Anomalies

Data on amphibian morphological anomalies (malformations and deformities) were collected in all surveys when individuals could be caught by hand (VES, dip-netting, bottle traps or pitfall traps) (Heyer et al. 1994). Captured postmetamorphic amphibians were examined for the presence of morphological anomalies. A field guide was used to identify the type of anomaly (Meteyer 2000). In some cases, body mass and snout-vent length (SVL) of the captured individuals were also measured to determine their age and condition. In addition, detailed descriptions and photographs were prepared, and the coordinates of the locality were also recorded using a hand-held GPS (Garmin Dakota 10, accuracy: 1-2 m).

3.2.2. Chapter 2

The data used here came from two field studies: (i) the long-term monitoring of restored grasslands and (ii) a regular baseline survey of natural grasslands as reference. In the post-restoration monitoring programme, our research group surveyed carabid beetles and ground-dwelling spiders on restored grasslands by using Barber traps. Two or three sampling sites were selected in each restored grassland in the year after restoration, resulting in a total of 35 sampling sites. Two years after restoration, a cattle-grazing experiment was initiated at 10 sites, where six traps were installed, resulting in a total of 60 traps. A mowing experiment was started two years after restoration at 10 other sites, where eight traps were installed, resulting in a total of 80 traps. Finally, 15 sites were not included in either experiment and two traps were installed at each of these sites, resulting in a total of 30 traps. Therefore, the total number of sampling sites was 35 and the number of Barber traps was 170. Sampling was continued for five years after restoration. Because restoration was carried out in four years (2005-2008), the number of traps was highest in 2009 and 2010 and decreased afterwards (e.g. monitoring was



discontinued in 2011 or year 6 on grasslands restored in 2005). Additionally, in 2015, 32 sites were resurveyed with two traps at each site (total $n = 64$ traps) in a chronosequence design. The total number of trap-days in the post-restoration monitoring was 64,468 in five years (2010–2013, 2015).

In the baseline survey of natural grasslands, two Barber traps were installed in each of the 49 natural grassland patches in a 1500-ha area not involved in restoration (Lengyel et al. 2016), resulting in a total of 98 traps. Sampling was conducted once in three years (in 2007, 2010, 2013 and 2016). Barber traps were operated as in the monitoring of restored grasslands and the total number of trap-days in the baseline survey was 44,310 (2010, 2013 and 2016).

In both studies, the Barber traps were plastic cups (0.5 l) containing 100 ml of 25% ethylene-glycol mixed with water and a few drops of detergent (Lengyel et al. 2013). The traps were sunk into the soil so that their rim was level with the soil surface and were covered lightly with a square-shaped wallboard (0.2×0.2 m) to keep out rainwater from the traps. Because many amphibians were found in the traps in 2010, a year with an extreme amount of precipitation (924 mm, i.e., 77% more than the average at the study site), our research group started to monitor the number of amphibian individuals caught inadvertently by the traps (Mester et al. 2015a). Pitfall trapping without a killing liquid is regularly used in field studies to survey amphibian species and communities (Greenberg et al. 1994; Sung et al. 2011; Dodd et al. 2012). In my study, the use of a killing liquid for invertebrates resulted in mortality of the majority of amphibians (see **Ethics statement**). In the two studies combined, the dataset included data from 84 sites and 268 traps between 2010 and 2016. Pitfall traps were operated usually from early May to early October and emptied once every three or four weeks or between five and seven occasions within a year. The total number of trap-days in the two studies was 108,778.



On each survey occasion, the traps were carefully checked, the invertebrate samples were collected, and all vertebrates (amphibians, lizards and small mammals) were examined and noted. Missing or damaged traps were also noted. Amphibians that were still alive in traps were immediately and thoroughly cleaned and set free in a safe habitat nearby. Dead amphibians were removed and safely discarded to avoid accidental poisoning of potential predators in all cases. Used liquid mixtures were also removed before re-filling the traps with new ones.

3.2.3. Chapter 3

To simulate natural disturbances a quasi-experimental design was applied in which three conservation treatments (controlled (prescribed) burning of reed in 2007 and 2009, and grazing) carried out in the marsh that were overlapped (see **Appendix 2**). The exact position and size of the areas that were burned and/or grazed were located by a field survey in March 2010 in which the borderlines of managed and unmanaged areas were recorded using a PDA (ASUS MyPal A639, accuracy: 2-3 m). Six management combinations were created in order of increasing disturbance intensity: (i) unmanaged (control), (ii) grazed and unburned (fire control), (iii) ungrazed and old-burned (burned in 2007), (iv) grazed, old-burned, (v) grazed, newly burned (burned in 2009), and (vi) grazed, burned in both years (2007 and 2009). In all six treatment combinations five transects were randomly selected as sampling units (total $n = 30$). First, starting points of the transects were randomly selected and then the orientation of the transects (length: 100 m) were also randomly determined. To lessen spatial non-independence transects were positioned at least 100 m from each other.

To study the effects of conservation treatments on anurans and their habitats call detection monitoring combined with VES was used while walking along the sampling units ($n = 30$ in 2010, $n = 25$ in 2011). The order in which transects were surveyed was determined randomly. Frogs



detected visually within 2 m and heard within 25 m either left or right from the survey track were recorded. To keep these distances accurate reference points were marked in each transects or in few cases, exact distances of observed individuals were measured. If the accurate number of calling frogs could not be certainly specified due to overlapping calls in a chorus (Wisconsin index (WI): > 2) counts of calling anurans were categorized (WI = 2; 5-10, 10-15, etc., WI = 3; 0-10, 10-20, etc.) (Paloski et al. 2014). If identification of a calling *Pelophylax* individual was uncertain it was recorded as *Pelophylax* spp. The study was implemented in mid-April the peak breeding period for most anurans (Puky et al. 2005; Glandt 2015).

To measure the effect of conservation management on reed four variables were recorded. First, reed cover was estimated as the proportion of transect length covered by reed in each transect. The four values were then averaged for the entire transect length to achieve mean reed cover (%). This variable was then arcsine-transformed to obtain normal distribution. Second, to measure the complexity of reedbeds, the coefficient of variation (CV) in reed cover was estimated using the standard deviation divided by the mean reed cover of the transects. Third, to study in detail how the last fire (2009) affected the reed properties, the density of reed was also measured in April 2010 by counting the number of old (dry) and new (green) reed stems in a 40 cm diameter circle ($\sim 0.126 \text{ m}^2$) held at a height of 1 m and 1m away in a randomly chosen direction at three internal points in all transect (at 25, 50 and 75 m). The latter two variables were also averaged for each transect.

The following environmental variables which may affect the observed activity and number of frogs were recorded: water temperature, water depth, and three weather variables. The depth (accuracy: 5 cm) and temperature of water were measured at five points (0, 25, 50, 75, and 100 m) in each transect. Wind speed, air temperature, and cloud cover (on a scale of 1 to 5) were also measured at three internal points (0, 50, and 100 m) along each transect. Environmental variables recorded at several



points along the transect were averaged for further analyses using one transect as one data point.

To measure vegetational changes, the research group sampled plants at 56 locations which were positioned randomly in the marsh before the first prescribed fire in June 2007. All flowering plants were recorded at each sampling point, and the cover of every determined species was estimated in a randomly placed 2×2-m plot. 30 of the 56 locations were burned in September 2007 and subsequently grazed in autumn 2007 and spring 2008, thus these sampling points were resurveyed using the same method in June 2008 for a before-after comparison.

Surveys on amphibians were implemented in 2011 and 2011. Unfortunately, due to the extreme drought weather in 2012 the HNP Directorate led the entire marsh to dry out to simulate weather extremes, and to carry out a dredging in 2013 located in the south-eastern part of the marsh. Moreover, effects of applied conservation actions were already weak in the second year after the managements due to the intensive rejuvenation of reed by the third spring (2012). Thus, the survey could not be continued.

The changes in the cover of reed and other plants may influence detection probability of anurans if detectability decreases with increasing reed cover. Thus, two studies were carried out to test this theory. First, in the early summer of 2010, 12 transects (in total 480 m) were surveyed other than the 100-m study transects for a total of 124 minutes in unmanaged reedbeds to make sure that no anurans were missed due to lower detection probability. The intensive search for frogs resulted in only nine specimen (one adult Edible Frog *Pelophylax kl. esculentus* L. 1758, five juvenile *Pelophylax* spp. individuals, and three adult *B. bombina*). Second, nighttime call detection monitoring was performed (Dodd et al. 2012) six times from late-March to early-June in 2011 in conjunction with the regular transect survey. If detection probability was lower in dense reedbeds, there should be no correlation or there should be a negative



correlation between the number of visually observed frogs and call intensity. As the Wisconsin index for anuran call intensity (Royle 2004) showed positive correlations with the number of visually found individuals (see Mester et al. 2015b), and the number of visually observed anurans and frogs detected by their calls in the regular transect surveys showed positive correlations, thus, detection probability was less of an issue (Mester et al. 2015b).

3.2.4. Chapter 4

To monitor the 47-km section of Main Road 33 running E-W across the Hortobágy region road transect method was used (Puky 2001a). To measure both early (early March to mid-March) and late (late March to early April) migration peaks the surveys were carried out twice in a year (2013-2017). Each surveys were implemented before, during or after the first heavy rainfalls, when the weather was warm (on average 15°C) and humid (on average 4 mm of rain), which conditions trigger amphibian migration (Semlitsch 2008; Garriga et al. 2017). Surveys were started one or two hours after sunset to catch the migration intensity peak and to prevent being blinded by the head-on traffic (Hels & Buchwald 2001). All surveys were carried out using vehicles because of the long distance and the relatively short available time window in which each surveys have to be completed (Colino-Rabanal & Lizana 2012). The survey speed was on average 30-40 km/h which ensures optimal detection probability with two or three observers (Garriga et al. 2017). If the head-on traffic was intense or during a heavy rainfall the survey speed was decreased to 15-20 km/h. In 2012, the exact location and length of the fences on either side of the road were also determined.



3.3. Data Analysis

3.3.1. Chapter 1

The faunistical and morphological anomaly data were sorted using MS Excel sheets. 2.5 and 10 square kilometer Universal Transverse Mercator (UTM) grids (see **Appendix 3**), which are frequently used resolutions in several ongoing monitoring projects, provided by Bird Life Hungary were used for UTM data to support further studies (Szép & Nagy 2001). Exact observation locations were collected and compiled using Garmin BaseCamp 4.5.2, and data were analysed in QGIS 2.16.2.

3.3.2. Chapter 2

Response variables in these analyses were species abundance (number of individuals caught per species), species richness (number of species) and total abundance (number of all individuals) per trap. Abundance was calculated for six species and one species complex: Smooth Newt (*Lissotriton vulgaris* (L. 1758)), *T. dobrogicus*, *B. bombina*, Common Spadefoot (*Pelobates fuscus* Laurenti, 1768), *Bufo bufo*, *H. arborea* and *Pelophylax* spp. individuals combined. Data from sampling occasions within a year were pooled to calculate response variables, resulting in one datapoint for one trap in each year. Independent variables included both local factors (restoration conditions) and landscape factors (proportion of major habitat types around the sampling sites). Restoration conditions were the type of the last crop before restoration (alfalfa, cereal, sunflower), the seed mixture used in restoration (alkali, loess) and restoration age (number of years since restoration, with 0 indicating the year of restoration and 1 indicating the first year after it).

To measure the effects of the landscape structure the proportion of eight major habitat types (marshes, meadows, grasslands, wooded areas, arable lands, residential areas, artificial ponds, and canals) were



calculated using circular buffers with a radius of 500 m around each trap. The chosen radius is based on the average reported post-breeding movement ranges of amphibians (Smith & Green 2005; Semlitsch 2008; Sinsch et al. 2012). To avoid multicollinearity in analyses, principal component analysis (PCA) on the eight variables was performed to reduce them into uncorrelated variables. One PCA for the restored grasslands and another one was also calculated for restored and natural grasslands jointly. In the PCA for restored grasslands, the first three components explained 63.9% of the variance, and PCA1 ('Elevation') correlated positively with wooded areas, arable lands and canals and negatively with wetlands (marshes and meadows), PCA2 ('Farms') correlated positively with residential areas, whereas PCA3 ('Dryness') correlated positively with grasslands and negatively with marshes (see **Appendix 4**). In the PCA for restored and natural grasslands, the first three components explained 67.8% of the variance, and PCA1 ('Naturalness') correlated positively with grasslands and negatively with arable lands, PCA2 ('Elevation') correlated positively with wooded areas and negatively with marshes, whereas PCA3 ('Farms') correlated positively with residential areas and negatively with meadows (**Appendix 4**). Thus, these principal components (PCA1-3) were used in the corresponding analyses.

To answer the research questions three analyses were performed. In step 1, generalised linear mixed-effects models (GLMMs) with repeated measures (GLMM-RM) were constructed to study the effects of grassland restoration and landscape structure on amphibians using all available data from five years (2010-2013, 2015) of restoration monitoring. GLMM-RMs were built using the 'glmer' function in the 'lme4' package of R (R Core Team 2016), in which Poisson error distribution and logit link function were specified. The models had two nested random effects; the first random effect was sampling site within area (restored grassland) to control for spatial non-independence, whereas the second one was area within years to control for temporal non-independence (Bates 2010). For



each of the nine response variables, first, the full model was fitted containing each of the three local and three landscape-scale fixed effects and the two random effects, then non-significant ($p < 0.05$) terms were manually removed to obtain minimal adequate models (MAMs). Significance levels were estimated for each model by the ‘Anova’ function in the ‘car’ package. For finite sample sizes it was checked whether the manual removal differed from a model selection approach based on Akaike’s Information Criterion (Burnham & Anderson 2002), and identical models were found in seven of nine cases. Further tests also confirmed the validity of manual removal (Supplementary Material), thus, MAMs obtained by manual removal were retained.

In step 2, short-term and mid-term effects of grassland restoration on amphibians were compared by performing similar analyses on data from two years when all sites were sampled (2010, 2015). In both analyses, GLMMs were used in a space-for-time substitution approach (chronosequence method) using the ‘lme’ function in the ‘nlme’ package of R to model the effects of the three local and three landscape-scale independent variables on the nine response variables. Significance levels were estimated for each model by function ‘Anova’. Sampling site was nested in area (restored grassland) as a nested random factor. In models for 2010, restoration age ranged from two years (restored in 2008) to five years (restored in 2005), whereas in models for 2015, it ranged from seven to ten years. The results from these two analyses were then evaluated to measure the short-term and mid-term effects of restoration on amphibians.

In step 3, differences between restored and natural grasslands were measured by using all data from the monitoring of restoration and the baseline survey available in 2010. GLMMs were used as described above (in step 1) for the nine response variables, with the only difference that a factor of ‘habitat type’ with two levels (restored and natural) was added. The random factor was sampling site nested within area (restored grassland or natural grassland patch). Although all plots were sampled



also in 2015, a similar comparison of restored and natural grasslands could not be made because data from the baseline survey were available only from 2013 and 2016, and differences in annual weather (Mester et al. 2015a) may have confounded such a comparison.

During fieldwork, some Barber traps were damaged or destroyed by cattle or emptied by unknown animals, resulting in differences in the number of trap-days among some areas. Thus, it was studied whether this difference influenced the results by repeating each of the three analyses above by excluding data from the traps that were damaged in one or more sampling periods within a year. As the results were qualitatively identical to the ones obtained by the full dataset, therefore, data from all traps were used in each of the three analyses.

For spatial calculations of landscape structure QGIS 2.16.2 was used. All analyses were implemented in the R statistical environment (version 3.2.3). To compare means in post-hoc testing of categorical factors, Tukey's HSD tests were used to calculate the significance of differences. Finally, the 'lsmeans' function in the 'lsmeans' package of R was used to calculate least-squares means and standard errors adjusted for the random effects.

3.3.3. Chapter 3

Analyses were implemented in three steps to measure the effects of applied conservation managements. First, general linear mixed-effects models (GLMEs) were used to study how conservation managements did impact reedbed properties (mean and CV reed cover, number of new and old reed stems). For mean and CV reed cover (available for 2010 and 2011), the model included the three managements (unburned in 2009 vs. burned in 2009, unburned in 2007 vs. burned in 2007, and ungrazed vs. grazed) as predictors and year as fixed effects. Transect identity was used as a random effect. To measure whether the impact of treatments differed between the years, all interactions between the management variables and



year were included. For the number of new and old reed (available only from 2010), GLMEs included the three treatments as fixed effects. GLMEs were built using the 'lme' function in the 'lme4' package of R. Moreover, species richness and total cover of flowering plants were compared before and after the first prescribed fire by Wilcoxon's matched pairs tests to more closely measure how did prescribed fire affect the diversity of marsh vegetation.

Second, the impact of reed properties was measured on the frog community. Response variables were species richness, total abundance, the total number of *Pelophylax* spp., and the number of *B. bombina*. For all four response variables, GLMMs were built using year, the management*year interactions, the two reed cover variables, five environmental variables as fixed effects, and transect identity as a random effect. GLMMs were fitted using the 'glmer' function (R Core Team 2016).

Third, effect of conservation managements was directly analyzed on species richness and total abundance of anurans. GLMMs were built again for four response variables (species richness, total abundance, total number of *Pelophylax* spp. and *B. bombina* abundance) to interpret their variation based on year, the three management variables, interactions between management and year, and the five environmental variables.

All biologically meaningful interactions and main effects were included in the full model of each steps. In GLMMs Z-statistic and its associated level of significance for each parameter in the model summary were used. Non-significant ($p > 0.05$) interactions and effects were then removed using backward stepwise method to achieve MAMs (Crawley 2007), which were then applied to measure management effects and to calculate coefficients. When management significantly ($p < 0.05$) influenced response variables means were compared by performing t-tests using Welch's adjustment of the degrees of freedom when variances were uneven. R 3.1.1 was used in this study (R Core Team 2016).



3.3.4. Chapter 4

Repeated measures analysis was used to evaluate the spatial and temporal variation in amphibian road mortality, the effects of surrounding habitat structure, and the effects of mitigation measures installed by the HNP in 2012. The basic unit for all analyses were 1-km sections of the 47-km section of Main Road 33. GLMM-RMs were performed using the 'lme' function in the 'nlme' package of R, in which the response variable was the number of individuals counted, whereas the independent variables were fix factors (year, season, proportion of fenced section, tunnel and the EPMS), environmental variables (proportion of habitat types), and transect identity was the random effect. Models were fitted in R 3.2.3 (R Core Team 2016).

Habitat structure along the studied road was characterised by digitising habitat patches within 750 m on each side of the studied 47-km section of Main Road 33 based on a botanical GIS layer provided by the HNP. The total number of habitat types which were identified previously using Google satellite and botanical layers were reduced from 19 to 13 by joining similar ones. A vector layer was then created containing a total of 1740 habitat patches (polygons) across the 47 kilometers. Next, 500-m-wide buffers were created for each 1-km road section by considering the average migration and dispersal range of local amphibians (Smith & Green 2005; Semlitsch 2008; Sinsch et al. 2012). To minimise spatial auto-correlation, a modified buffer layer was made in which overlapping semicircle endings were deleted to result in 1×1-km rectangular buffers (area: 100 ha) for each 1-km section. Areas lying farther than 500 m from the road were removed from the analysis. Finally, the area/proportion of each habitat type were calculated in each buffer. For these steps QGIS 1.8.0 and OpenLayers plugin were used.

Fence lengths recorded on the two sides of the road for each 1-km section were averaged to calculate the proportion of fenced sections. For



proportional variables (habitat types and fenced sections) arcsine-transformation were applied to obtain normal distribution.

The following equation was used to estimate amphibian roadkill probability (Hels & Buchwald 2001; Gibbs & Shriver 2002):

$$P_{\text{killed}} = e^{-Na/v}$$

in which P_{killed} is the probability of the mortality of one individual crossing the road at right angles, e is the Euler–Mascheroni constant, N is traffic intensity (vehicle/minutes), a is the 'killer zone' which is equal with twice the average tyre width plus two times the average SVL of the individual crossing the road (both in meters), and v is the average movement velocity of an individual. Traffic intensity data were obtained from the Hungarian Public Road company (HPR) and the "KIRA" GIS databases (HPR 2017). Considering that 72% of the vehicles are cars and the remaining are mainly trucks and buses along the studied section of Main Road 33 (HPR 2017), the average tyre width was estimated at 220 mm. Data on average SVL and migration velocity were available for common local amphibians from (Hels & Buchwald 2001; Arnold & Ovenden 2005; Glandt 2015). Although 'killer zone' can be much wider in the case of large and fast vehicles such as trucks, this equation estimates 'the best scenario' in which an amphibian individual is continuously crossing the road and the most frequent vehicle type are cars and slower agricultural machines.

To characterise traffic intensity, traffic data was used from all 16 years available (2000-2015). Traffic intensity data were available for four road sections monitored by the HPR that almost covered the entire Hortobágy section of Main Road 33. Data from HPR were available from three time periods (daytime, evening, nighttime) for every surveyed sections. To calculate traffic intensity, the data were averaged for the entire Hortobágy section, for all the three time periods, and for all years (2000-2015). Estimated road mortality probabilities were calculated in R 3.2.3 (R Core Team 2016).



4. Chapter 1 - Reducing Data Gaps: Herpetofauna of the Egyek-Pusztakócs Marsh System

4.1. Introduction

The loss of amphibian populations is a global process (Beebee & Griffiths 2005). In Europe, their decline is mainly caused by agricultural intensification and increasing urban sprawl (Sanderson et al. 2002; Hamer & McDonnell 2008). Beside the aquatic habitats amphibians also require suitable terrestrial habitats surrounding and connecting wetlands (Balas et al. 2012; Mester et al. 2015a).

In the Carpathian Basin large and natural lowland wetland and grassland systems still exist providing good quality habitats for 12 amphibian species (Puky et al. 2005; Gubányi et al. 2010). However, these habitats are also altered by human activity (Vörös et al. 2015), for example by the intensive land use on the fertile soils along the Danube and Tisza rivers. Protection, restoration and conservation of these habitats thus have played an important role in amphibian conservation since the first national park in Hungary, the HNP was established.

In Hungary, the first countrywide herpetofaunistical survey was started in 1987 focussing on the Danube, Tisza, Szamos and Ipoly rivers (Puky 2001b; Puky & Fodor 2002; Puky 2007). The first herpetological atlas was then published in 2005 (Puky et al. 2005), which reported the results of most of the surveys carried out in the 20th century using UTM grids (Szép & Nagy 2001). Further large-scale surveys on herpetofauna also used UTM grids (Bakó & Korsós 1999; Szép et al. 2011). At the same time, the atlas identified several areas with knowledge gaps in Hungary, and the Hortobágy region was one of them (Bakó & Korsós 1999; Puky et al. 2005; Gubányi et al. 2010). Although Dely (1981) and Endes (1988) listed 11 lowland amphibians (*L. vulgaris*, *T. dobrogicus*, *B. bombina*, *P. fuscus*, *B. bufo*, *B. viridis*, *H. arborea*, the Moor Frog (*Rana arvalis* Nilsson, 1842), *P. ridibundus*, *P. kl. esculentus*, *P. lessonae*)



occurring in the Hortobágy region, the abundance and ecology of these species remained unclarified.

Amphibians can be used as indicators of the status and condition of both their aquatic and terrestrial environments due to their biphasic life cycle (Vitt & Caldwell 2014). Negative processes in an ecosystem such as water contamination can be a factor which causes morphological abnormalities (anomalies) in amphibians (Blaustein & Johnson 2003; Boone et al. 2007). Johnson et al. (2001) distinguished three types of anomaly in amphibians: (i) anomalies, that include any traumatic or gross developmental deviation from the normal range of the morphological variation, (ii) malformations, when abnormal development causes permanent structural defects, and (iii) deformities, when an organ or a structure which was developed correctly is modified by a mechanical factor (e.g. unsuccessful predation).

Anomalies occur in association with a variety of cofactors. Pesticides (Hayes et al. 2006) or other chemical contaminants (Sessions et al. 1999) often result in the disruption of the endocrine system, the development of malformations, and the occurrence of extra limbs. UV-B radiation increases developmental abnormalities and reduces survival mainly in larval anurans (Pahkala et al. 2001). Limb malformations can be linked to trematodes (Johnson et al. 2002; Blaustein & Johnson 2003). Finally, unsuccessful predation attempts by aquatic predators (e.g. larval dragonflies, crayfish, etc.) can cause injury in the developing limbs, which may resemble partially regenerating malformations in post-metamorphic anurans (Bowerman et al. 2010; Johnson & Bowerman 2010).

The frequency of anomalies in an amphibian population can reach 80-90% of all individuals due to the factors listed above and their synergistic or interactive effects (Johnson & Bowerman 2010). In contrast, the endemic, expected level (background frequency) of anomalies was estimated between 0 and 5% for postmetamorphic frogs in wild populations in natural habitats (Vershinin 1989; Ouellet et al. 1997;



Piha et al. 2006; Puky 2006; Reeves et al. 2013). For adult amphibians, this background frequency can be much less than 1% (Piha et al. 2006; Puky 2007; Henle et al. 2017).

Considering that the use of fertilizers and pesticides is increasing globally due to the agricultural intensification (Tscharntke et al. 2012), it can be expected that amphibian decline will further accelerate in regions where they are exposed to these chemical contaminants. Based on this contexture, large and undisturbed protected areas may serve as a refuge in which healthy and large populations of amphibians exist. However, in Europe, agricultural activities are present in several protected areas, thus amphibians can be exposed to risks of chemical pollution. Although an evaluation of such risks within Special Areas of Conservation (SACs) established for amphibians of European conservation importance based on the Habitats Directive of the European Union found low risks for globally threatened and European priority species (Wagner et al. 2014), there were several exceptions. For instance, the risk of habitat contamination by pesticides was above average for eight amphibians, including *B. bombina* and *T. dobrogicus*, even in such protected areas, and these species were thus recommended for monitoring the future effects of contamination (Wagner et al. 2014).

Despite of the decreasing use of agrochemicals on terrestrial and aquatic habitats in the EPMS after the HNP was established, it remained still relatively high on croplands before the grassland restoration was launched in 2005 (Lengyel et al. 2012). As a result of the restoration, the proportion of croplands decreased, grassland corridors and buffer zones were created between marshes and croplands, and agricultural pollution and disturbance were reduced in the restored areas (Lengyel et al. 2012).

The systematic study of amphibian anomalies started in Hungary in the early 1990s. The first systematic surveys on the Danube, Tisza, and Ipoly rivers between 1987 and 2001 found anomalies in 13 species (Puky & Fodor 2002; Puky 2006; Puky 2007). However, little information was available on anomalies in lowland wetlands such as the EPMS.



4.2. Results

Amphibian fauna of the study site

In total, 14,362 individuals of 11 amphibian species were observed (**Table 1**). *Bombina bombina* was the most common (7182 individuals). The *Pelophylax* complex and three other species (*T. dobrogicus*, *H. arborea*, *P. fuscus*) were also common. Two species listed in Annex II of the EC Habitats Directive, the *B. bombina* and *T. dobrogicus* occurred in large numbers.

Bombina bombina and *H. arborea* occurred in 14 of the 15 2.5×2.5-km UTM squares covering the EPMS and all species detected occurred in 7 or more UTM squares (**Table 2, Appendix 3**). All the 11 species were recorded in three (96A1, 96C1, 96C2) of the 15 2.5×2.5-km UTM squares covering the EPMS and in two (DT96, DT97) of the four 10×10-km UTM squares covering the EPMS. Whilst all the 11 species and 71% of the individuals occurred in those 2.5×2.5-km UTM squares that were at least 60% covered by the EPMS ($n = 6$, highlighted in bold in **Table 1**), all the 11 species were also detected when the squares that were less than 60% were used only.



Table 1. Species abundance and richness, total abundance, number of survey days and percent cover of the EPMS in 2.5×2.5-km UTM squares. UTM cells with EPMS cover above 60% are in bold. Here ‘*Pelophylax* spp.’ indicates individuals for which species identification was not possible, and ‘Water frogs total’ indicates the summed number of *Pelophylax* individuals.

2.5×2.5-km UTM square																
Species	86C3	87D4	96A1	96A2	96A3	96A4	96C1	96C2	96C3	96C4	97B1	97B2	97B3	97B4	97D4	Total
<i>L. vulgaris</i>	0	0	23	5	2	0	40	54	106	0	19	52	1	0	0	302
<i>T. dobrogicus</i>	0	8	181	36	37	24	206	75	187	0	145	599	85	69	0	1652
<i>B. bombina</i>	121	15	720	425	795	596	1892	853	341	0	168	859	190	157	50	7182
<i>P. fuscus</i>	8	31	68	4	103	66	108	69	71	0	78	745	30	55	0	1436
<i>B. bufo</i>	1	0	0	1	0	9	6	31	0	0	0	4	0	1	0	53
<i>B. viridis</i>	1	0	1	0	0	4	14	14	0	0	0	0	2	20	0	56
<i>H. arborea</i>	51	0	270	180	482	379	105	179	56	1	1	123	40	38	2	1907
<i>R. arvalis</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	4
<i>P. lessonae</i>	10	0	25	8	52	20	32	192	0	0	1	28	0	0	0	368
<i>P. ridibundus</i>	0	0	8	7	1	5	7	39	0	0	16	8	0	0	0	91
<i>P. kl. esculentus</i>	0	0	10	0	12	15	30	37	4	0	15	6	0	4	0	133
<i>Pelophylax</i> spp.	0	0	14	6	259	88	325	439	0	0	0	39	0	3	5	1178
Water frogs total	10	0	57	21	324	128	394	707	4	0	32	81	0	7	5	1770
Species richness	6	3	10	8	8	9	10	10	6	1	8	9	6	7	3	
Total abundance	192	54	1321	672	1743	1206	2765	1982	765	1	443	2463	348	347	60	
Survey days	7	6	11	7	25	17	28	27	18	1	13	25	12	14	2	
EPMS (%)	19	28	100	41	90	62	91	43	69	3	44	100	9	38	4	



Table 2. Occurrence of amphibian species, species richness (with literature data shown in parentheses), number of survey days and percent cover of the EPMS in 10x10-km UTM squares, EC Habitats Directive Annex listings, and global population trends (Temple & Cox 2009). Symbols: +: detected in this study, (+): probable occurrence based on this study, *: occurrence from Puky et al. (2005), **: occurrence from other literature source.

Species	10×10-km UTM square				Annex	Trend
	86	87	96	97		
<i>L. vulgaris</i>	(+)	*	+**	+	-	stable
<i>T. dobrogicus</i>	(+)	+	+*/**	+*/**	II	decreasing
<i>B. bombina</i>	+**	+	+	+*/**	II	decreasing
<i>P. fuscus</i>	+**	+	+**	+	IV	decreasing
<i>B. bufo</i>	+**	*	+**	+	-	stable
<i>B. viridis</i>	+**	*	+	+	IV	decreasing
<i>H. arborea</i>	+	(+)	+**	+	IV	decreasing
<i>R. arvalis</i>	-	+	+**	+	IV	stable
<i>P. lessonae</i>	+**	+	+**	+	IV	decreasing
<i>P. ridibundus</i>	(+)	*	+**	+	V	increasing
<i>P. kl. esculentus</i>	*	*	+	+	V	decreasing
Species richness	6 (2)	10 (10)	11 (4)	11 (10)		
Survey days	6	6	46	27		
EPMS (%)	1.8	11.9	31.1	12.1		



Morphological Anomalies

Although a total of 5,596 individuals of 11 species were examined between 2010 and 2016 (**Table 3**), only 16 individuals of four species and one species group (*Pelophylax* spp.) showed any morphological anomalies, corresponding to a total frequency of 0.3%. All anomalies were deformities and no evidence of malformation was found. Mass deformities (i.e. morphological anomaly frequency above 30%) was not found, and the distribution of amphibians showing anomalies was sporadic throughout the EPMS.

The most common types of anomaly were ectro- and hemimelia (i.e. the partial or complete loss of the hindlimbs) found in six *B. bombina*, three juvenile *Pelophylax* water frogs, one adult *H. arborea*, and one adult *P. fuscus*. A juvenile *B. bombina* of these showed multiple deformities (partial loss of a hindlimb and a forelimb). All ectro- and hemimelia were asymmetrical. Five *T. dobrogicus* showed deformities. One subadult newt had a partial loss of its tail, another had a partial loss of its left hindlimb, one adult newt had a complete loss of its left forelimb, and one subadult *T. dobrogicus* had multiple deformities (partial loss of right forelimb and tail).

On 16 September 2010, a *T. dobrogicus* individual was found with a secondary tail (tail bifurcation) in a Barber trap, south of the village of Egyek (47°36'42.45"N, 20°54'4.61"E; 86 m a.s.l.). A vertebra of the secondary tail was attached ventrally to the vertebral column with a pseudo-symphysis (Henle et al. 2012).



Table 3. Number of individuals examined and anomalies found in species detected in the EPMS between 2010 and 2016.

Species	Number of juveniles (= 1st year)/adults and subadults (\geq 2nd year) examined								Anomaly juv./ad.
	2010	2011	2012	2013	2014	2015	2016	Total	
<i>L. vulgaris</i>	2/69	0/107	4/27	2/0	-	-	2/2	10/205	—
<i>T. dobrogicus</i>	2/722	0/12	2/37	0/1	-	-	16/84	24/856	0/5
<i>B. bombina</i>	1292/1198	0/50	0/224	0/122	0/50	0/5	30/72	1322/1721	3/3
<i>P. fuscus</i>	0/78	0/71	1/25	0/32	0/2	0/40	26/188	27/436	0/1
<i>B. bufo</i>	5/0	—	—	—	-	0/1	-	5/1	—
<i>B. viridis</i>	0/7	—	—	—	0/4	-	-	0/11	—
<i>H. arborea</i>	7/3	—	0/21	0/12	0/4	0/1	1/1	8/42	0/1
<i>R. arvalis</i>	—	—	0/2	—	1/0	-	-	1/2	—
<i>Pelophylax</i> spp.	742/0	2/0	34/2	13/23	2/105	-	2/0	795/130	3/0
Total	2050/2077	2/240	41/338	15/190	0/168	0/47	77/347	2192/3404	6/10



4.3. Discussion

This study presented the first comprehensive data on the amphibian fauna of the EPMS. Except for *R. arvalis* which has the nearest known locality in the floodplain forests along the River Tisza (Marián 1963; Puky et al. 2005), all of the lowland amphibians that can be found in Hungary (**Table 2**) were detected.

The study also reports new occurrence data in UTM grids for all species observed. Most of the new occurrence records were from the DT86 and DT96 10×10-km UTM cells (**Table 2**). Although *T. dobrogicus* was found in only three of the four 10×10-km UTM squares, it is likely that it also occurs in DT86. In the DT97 cell which covers the large Csattag-lapos marsh and several smaller wetlands (see **Appendix 3**), all the 11 species were known previously (Puky et al. 2005; Gubányi et al. 2010). In contrast, in the DT96 square which includes seven large wetlands (**Appendix 3**), only four amphibian taxa were known previously (Puky et al. 2005; Gubányi et al. 2010). The DT86C3 and DT86C4 cells cover the Hagymás-fertő marsh (also see **Appendix 3**), where five additional species were observed along with the previously known *H. arborea* and *P. kl. esculentus*. In the Hagymás-fertő marsh, *T. dobrogicus* and *L. vulgaris* can also occur as they were present in the nearby wetlands, particularly in Hagymás-lapos marsh. The Marsh Frog (*Pelophylax ridibundus* Pallas, 1771) can also occur here as Hagymás-fertő marsh provides deep and permanent water. Although *H. arborea* was not observed in the DT87 square, it probably occurs in this UTM cell because of the proximity of large marshes and the relatively high dispersal ability of the species (Smith & Green 2005).

The survey on amphibian morphological anomalies detected no malformations and a low frequency of deformities in the EPMS. This frequency (0.3%) is far below the high prevalence of anomalies reported in several North American studies (reviewed in Blaustein & Johnson 2003; Sessions 2003). The results have conservation relevance, because



the two most abundant species of the EPMS, the *B. bombina* and *T. dobrogicus* are listed as priority species in Annex II of the EC Habitats Directive and were also predicted to have high risk of pesticide exposure (Wagner et al. 2014). In addition, all but two of the 11 species detected have a decreasing global population trend and are included either in Annex II or IV of the EC Habitats Directive (**Table 2**) (Temple & Cox 2009).

Most of the deformities occurred in common amphibians of the EPMS (*B. bombina*, *Pelophylax* spp., *P. fuscus*) (**Table 3**), which is similar to what Puky (2006) reported based on a study of 50,000 individuals in Hungary. *Bombina bombina* and *Pelophylax* spp. can show numerous anomalies (Puky & Fodor 2002; Puky 2006), but other than ectro- and hemimelia, no other anomalies were found in the EPMS. At the same time, only a few paper report morphological anomalies in *T. dobrogicus* (Puky 2006; Henle et al. 2012). Similar to what found in *B. bombina*, ectro- and hemimelia were also observed in *T. dobrogicus* in almost all cases. These anomalies along with ectrodactyly, are the most frequent type of morphological anomalies in Hungary (Puky 2007). Finally, ectromelia was also detected in an adult *H. arborea*.

Because the most frequent morphological anomaly was the loss of hindlimbs, it is possible that the observed deformities were related to unsuccessful predator attack (Bowerman et al. 2010; Johnson & Bowerman 2010). Potential predators can be birds (e.g. egrets, herons, storks, the Kestrel *Falco tinnunculus* L. 1758, and the Red-footed Falcon *Falco vespertinus* L. 1766), reptiles (the European Pond Terrapin *Emys orbicularis* L. 1758), predatory fish, and aquatic arthropods (coleopterans, hemipterans, larval odonates, crayfish). Even though signs of injuries (e.g. because injuries to the developing animal (tadpole, larvae) may not show obvious scarring and wound signs once the animals have metamorphosed) were not observed, predation is a likely explanation for the deformities found. The tail bifurcation observed in a *T. dobrogicus* individual is the first evidence of this rare type of morphological anomaly



in this species (Henle et al. 2012). However, the exact cause is unknown (Henle et al. 2012). The attachment type of the secondary tail may indicate that the dorsal spine was injured, for example after an unsuccessful predation attempt by an avian predator, thus, it can be explained as a hyperregeneration (Henle et al. 2012).

It is unlikely that the observed deformities could be explained by exposure to retinoid compounds or infection by *Ribeiroia* parasites, which are often the causal agents of malformations (Johnson et al. 2002). Because Trematoda, Nematoda and Acanthocephala parasites found in amphibians was previously reported in the Hortobágy region (Edelényi 1974; Murai et al. 1983), parasites as possible local factors of morphological anomalies cannot be excluded (Herczeg et al. 2016), especially if their effect is combined with other factors (Reeves et al. 2010; Hof et al. 2011). Finally, because all limb losses of frogs were asymmetrical, the role of increased UV-B radiation could be marginal, which typically causes symmetric anomalies (Pahkala et al. 2001).

Amphibian malformations are reported to occur due to exposure to agrochemicals such as nitrogenous fertilizers (Rouse et al. 1999) and pesticides (Hayes et al. 2006). The result of this study is similar to what Piha et al. (2006) found in Common Frogs (*Rana temporaria* L. 1758) in agricultural habitats of Finland, and concluded that at the current levels of application, agrochemicals were not a threat to amphibians. In addition, low frequencies of anomalies were also found in Green Frogs (*Rana clamitans* Latreille, 1801) in southwestern Michigan (Gilliland et al. 2001) and in Japanese Fire-bellied Newt (*Cynops pyrrhogaster* Boie, 1826) exposed to chemical contaminants (Meyer-Rochow & Asashima 1998). Thus, low frequencies of deformations and no observed malformations in this study may support the conclusions of the above cited studies that agrochemical use did not represent threats to the morphological development of local amphibians. However, agrochemicals can be a threat to amphibians even if few morphological anomalies are observed, for example, if their effects are lethal. However,



we did not find any dead individuals at the study site. Thus, it remains uncertain whether the low frequency of deformities found reflect reduction in the agrochemical use due to earlier protection of the EPMS (since 1973) or to the more recent (2004-2013) landscape-scale rehabilitation and conservation managements.

In summary, large and apparently healthy amphibian populations were found in the EPMS. This study provides an example that large and healthy populations of amphibians can exist in large protected wetland complexes restored and managed for biodiversity conservation. These results are relevant because amphibians provide ecosystem services (e.g., mosquito control) and vital food resources for a number of aquatic birds and birds of prey for which the Hortobágy is famous and became protected more than 40 years earlier.



5. Chapter 2 - Compensating Habitat Loss: The Effects of Habitat Restoration on Amphibians

5.1. Introduction

Habitat loss is one of the major threats to biodiversity and ecosystem services (Sanderson et al. 2002). The areal decrease of natural grasslands is an important component of this process (Bakker & Berendse 1999). Grasslands are important terrestrial habitats, which provide a wide range of ecosystem services (Tilman et al. 2006; Isbell et al. 2011). Slowing down the rate of grassland loss is thus a considerable task that conservation has to face.

Although the global decline of amphibians has been well-known since the early 1990s (Blaustein et al. 1994; Poppy et al. 2000), the factors and their interactions which trigger the decline are poorly understood (Beebee & Griffiths 2005; Nyström et al. 2007; Blaustein et al. 2011). However, it has become clear that habitat loss plays a key role in amphibian decline (Lehtinen et al. 1999; Cushman 2006). Habitat restoration can thus in theory contribute to counterbalancing or at least reducing the rate of habitat loss. Several studies focused on the effects of restoration on amphibians in aquatic habitats (Petranka et al. 2007; Rowe & Garcia 2014; Klaus & Noss 2016), however, only a few deal with the effects of restoration on amphibians in terrestrial habitats (Smith & Sutherland 2014). Terrestrial habitats should be also important for conservation due to the biphasic life cycle of amphibians. Lowland grasslands, especially those that surround wetlands, can play important roles in maintaining the connectivity of amphibian populations and providing feeding and overwintering sites for amphibians (Wilgers & Horne 2006; Balas et al. 2012; Searcy et al. 2013; Larson 2014). As a result, the restoration of terrestrial habitats, and grasslands in particular,



can be important in amphibian conservation (Smith & Sutherland 2014; Sutherland et al. 2015).

Despite the potential importance of grassland restoration to amphibians, there are relatively few studies on its effect on amphibian populations, species and assemblages located in large lowland grassland and wetland complexes (Smith & Sutherland 2014). To fill this gap, I evaluated the effect of restoration on amphibians by using data on anuran amphibians (frogs and toads) caught in pitfall traps originally intended to sample ground-dwelling invertebrates (carabid beetles, spiders etc.).

5.2. Results

3021 individuals of seven amphibian taxa (255 *L. vulgaris*, 914 *T. dobrogicus*, 1360 *B. bombina*, 470 *P. fuscus*, 7 *B. bufo*, 7 *H. arborea*, and 8 *Pelophylax* water frogs) were found in Barber traps (baseline survey: 2010, 2013, 2016; restoration monitoring: 2010-2013, 2015) during 82,990 trap-days. All seven *H. arborea*, over 99% of *P. fuscus*, and more than 96% of *B. bombina* individuals were adults (over 2 years). 94% of *L. vulgaris* and 84% of *T. dobrogicus* individuals were immature efts (newts aged 1-3 years, Cogălniceanu & Miaud 2002), all the eight *Pelophylax* water frog individuals, and six of seven *B. bufo* individuals were juveniles.

5.2.1. Effects of grassland restoration and habitat structure

Data from traps operated in five years (2010-2013, 2015) in restored grasslands was analyzed. These traps caught 2526 individuals of seven amphibian taxa (228 *L. vulgaris*, 753 *T. dobrogicus*, 1065 *B. bombina*, 465 *P. fuscus*, 6 *B. bufo*, 7 *H. arborea*, and two *Pelophylax* water frogs).

The total abundance of amphibians was influenced by restoration age, last crop and seed mixture (**Table 4**). Significantly more amphibians



were caught in three to six-year-old restorations than in seven to ten-year-old ones (**Fig. 2A**).

Significantly more amphibians were found in former cereal and sunflower fields than in former alfalfa croplands (**Fig. 3A**). Finally, significantly more amphibians were caught in loess restorations than in alkali ones (**Fig. 4A**).

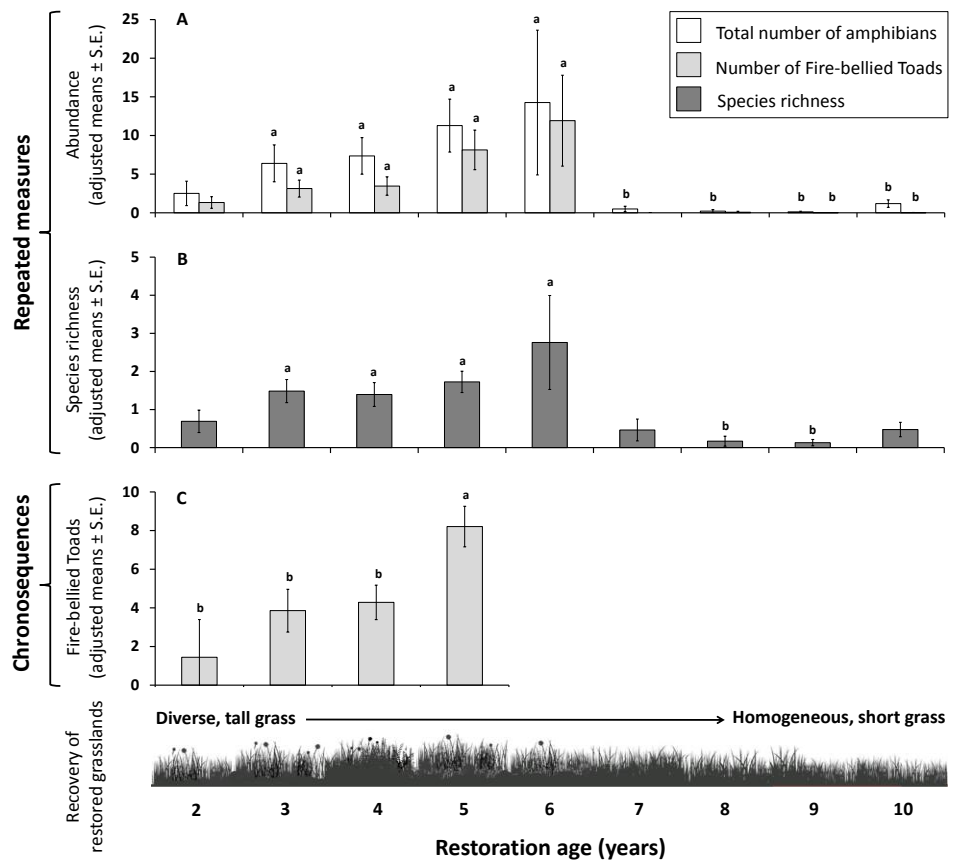


Figure 2. Dependent variables affected significantly by the age of the restored grasslands. Total number of amphibians with the number of Fire-bellied Toads (A), and species richness (B) using repeated measures method, and number of Fire-bellied Toads (C) using chronosequences along the restoration age. Different lowercase letters indicate significant differences (Tukey HSD test, $p < 0.05$).

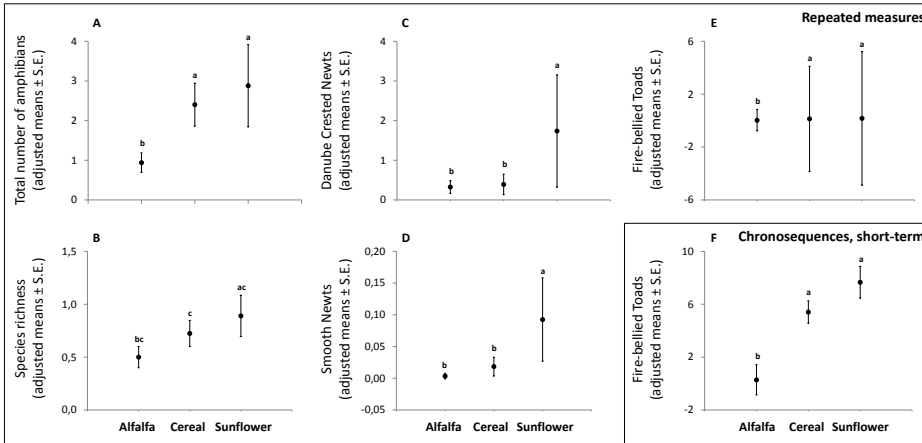


Figure 3. Dependent variables affected significantly by the last crop using two types of analysis approach. Total number of amphibians (A), species richness (B), number of Danube Crested Newts (C), number of Smooth Newts (D), and number of Fire-bellied Toads (E) using repeated measures method, and number of Fire-bellied Toads (F) using chronosequences among three types of crop history (alfalfa, cereal and sunflower). Different lowercase letters indicate significant differences (Tukey HSD test, $p < 0.05$).

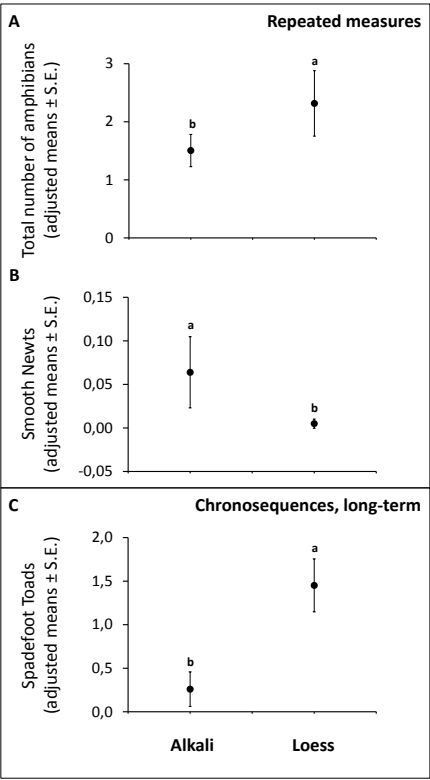


Figure 4. Dependent variables affected significantly by the seed mixture using two types of analysis approach. Total number of amphibians (A), and number of Smooth Newts (B) using repeated measures method, and number of Spadefoot Toads (C) using chronosequences between two types of seed mixture used (alkali, loess). Different lowercase letters indicate significant differences (Tukey HSD test, $p < 0.05$).



Species richness was influenced by restoration age and last crop (**Table 4**). Species richness was higher in restorations aged from three to six years than in restoration aged from seven to nine years (**Fig. 2B**). Former sunflower fields were more species rich than former alfalfa croplands, whereas former cereal fields did not differ from either of them (**Fig. 3B**).

The number of *T. dobrogicus* individuals was significantly influenced by last crop, and three PCAs (**Table 4**). Significantly more newts were caught in former sunflower fields than in cereal and alfalfa fields (**Fig. 3C**). Newt abundance was also significantly and negatively influenced by ‘Elevation’, and ‘Farms/cattle-pens’, and ‘Dryness’ (**Figs. 5A-C**), indicating more newts in lower-lying, wetter areas which are used as cattle pastures of local farms, with lower disturbance than in the higher-lying pastures.

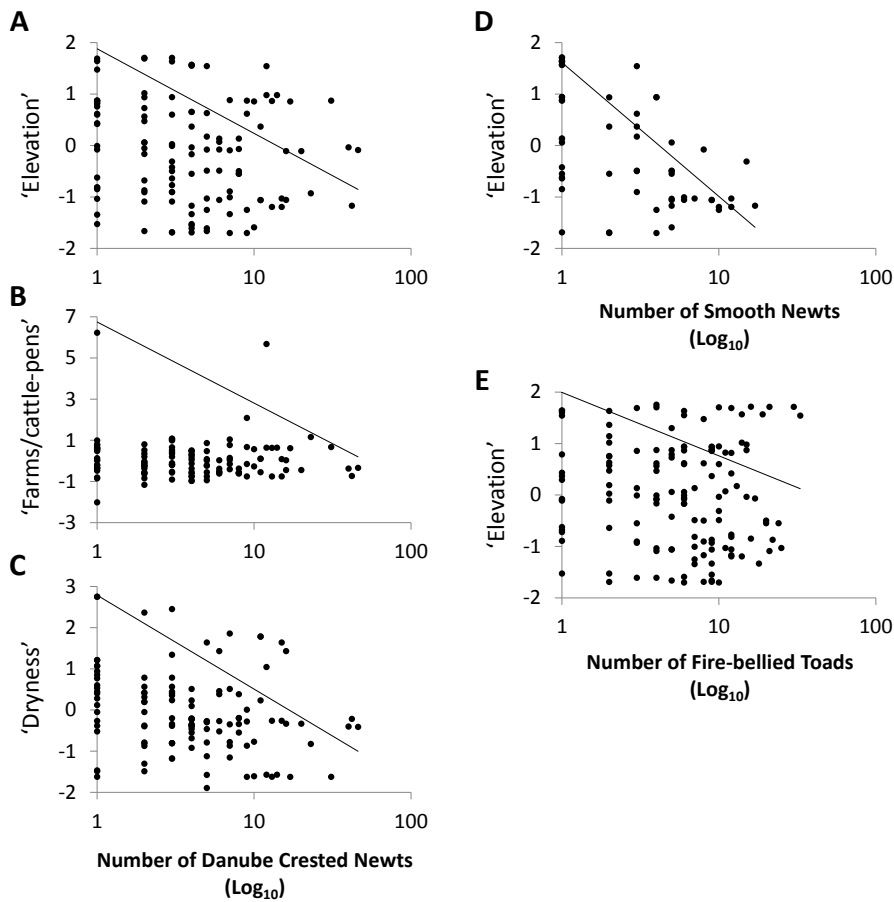


Figure 5. Correlations between the number of Danube Crested Newts and 'Elevation' (A), 'Farms/cattle-pens' (B), and 'Dryness' (C), the number of Smooth Newts and 'Elevation' (D), and the number of Fire-bellied Toads and 'Elevation' (E).

The number of *L. vulgaris* was significantly influenced by last crop, seed mixture and 'Elevation' (Table 4). The number of *L. vulgaris* individuals was higher in former sunflower fields than in cereal and alfalfa fields (Fig. 3D). Higher numbers of *L. vulgaris* individuals were caught in alkali restorations than in loess grasslands (Fig. 4B). Finally,



the number of *L. vulgaris* individuals correlated negatively with ‘Elevation’, indicating more individuals in lower-lying areas (**Fig. 5D**).

The number of *B. bombina* individuals was influenced by restoration age, last crop and ‘Elevation’ (**Table 4**). Post-hoc testing showed significantly fewer toads in nine-year-old restorations than in three to six-year-old ones, and in ten-year-old restorations than in five to six-year-old restorations, whereas the number of toads did not differ between nine and ten-year-old restorations (**Fig. 2A**). Traps located in former alfalfa fields caught significantly lower number of *B. bombina* individuals compared to the former cereal and sunflower croplands, which did not differ from each other (**Fig. 3E**). ‘Elevation’ correlated negatively with *B. bombina* abundance (**Fig. 5E**), indicating more toads in lower-lying restorations.

Independent variables did not influence *P. fuscus*, *B. bufo*, *H. arborea*, and *Pelophylax* spp. abundance.



Table 4. Minimal adequate generalized linear mixed-effects models testing the effects of restoration and habitat structure variables on abundance, species richness and number of *L. vulgaris*, *T. dobrogicus*, and *B. bombina* in restored grasslands.

Groups	Variables in final model	χ^2	p	Relationship/difference
Abundance	Restoration age	66.5	< 0.001	3 > 8,9 and 4-6 > 7-9 years old
	Last crop	12.4	0.002	sunflower = cereal > alfalfa
	Seed mixture	4.03	0.044	loess > alkali
Species richness	Restoration age	36.2	< 0.001	3-6 > 9 and 6 > 9 years old
	Last crop	6.47	0.039	sunflower > alfalfa
T. dobrogicus	Last crop	6.90	0.031	sunflower > cereal = alfalfa
	'Elevation'	25.6	< 0.001	negative correlation
	'Farms'	9.11	0.002	negative correlation
	'Dryness'	7.41	0.006	negative correlation
L. vulgaris	Last crop	20.8	< 0.001	sunflower > cereal = alfalfa
	Seed mixture	8.33	0.003	alkali > loess
	'Elevation'	23.4	< 0.001	negative correlation
B. bombina	Restoration age	29.8	< 0.001	3-6 > 9 and 5,6 > 10 years old
	Last crop	20.4	< 0.001	sunflower = cereal > alfalfa
	'Elevation'	12.3	< 0.001	negative correlation



5.2.2. Temporal differences among the effects of grassland restoration and habitat structure

Early stages of restoration

Data from 166 traps operated in restored grasslands in 2010 was analyzed in a chronosequence design. These traps caught 1446 individuals of six amphibian species (43 *L. vulgaris*, 596 *T. dobrogicus*, 856 *B. bombina*, 112 *P. fuscus*, 3 *B. bufo*, and 7 *H. arborea*).

Neither the total abundance, nor the species richness, nor the number of the two newt species (*L. vulgaris* and *T. dobrogicus*) were influenced by any of the independent variables (**Table 5**). The abundance of *B. bombina* was significantly influenced by restoration age and last crop (**Table 5**), and more individuals caught in five-year-old restorations than in younger restorations (**Fig. 2C**). Significantly more *B. bombina* individuals were caught in former sunflower and cereal croplands than in former alfalfa fields (**Fig. 3F**). Independent variables did not influence *P. fuscus*, *B. bufo*, and *H. arborea* abundance.

Late stages of restoration

Data from 64 traps operated in restored grasslands in 2015 was analyzed. These traps caught 43 individuals of two amphibian species (5 *B. bombina*, 38 *P. fuscus*).

Total abundance, species richness, *T. dobrogicus* and *B. bombina* abundance were not influenced by any of the independent variables (**Table 5**). However, the abundance of *P. fuscus* was significantly influenced by seed mixture (**Table 5**), indicating more *P. fuscus* individuals found in loess grasslands than in alkali restorations (**Fig. 4C**).



Table 5. Minimal adequate GLME models testing the effects of restoration and habitat structure variables on abundance, species richness and the number of *T. dobrogicus*, *B. bombina*, and *P. fuscus* using data from chronosequences of 31,374 and 8,192 trap-days for 2010 and 2015, respectively.

Time scale	Dependent variable	Variables in final model	χ^2	p	Relationship/difference
Short-term	Abundance	-			
	Species richness	-			
	<i>T. dobrogicus</i>	-			
	<i>B. bombina</i>	Restoration age	11.87	0.007	2005 > 2006-2008
		Last crop	21.33	< 0.001	sunflower = cereal > alfalfa
Long-term	<i>P. fuscus</i>	-			
	Abundance	-			
	Species richness	-			
	<i>T. dobrogicus</i>	-			
	<i>B. bombina</i>	-			
	<i>P. fuscus</i>	Seed mixture	11.19	< 0.001	loess > alkali



5.2.3. Differences among restored and natural grasslands

Data from 264 traps operated in restored and natural grasslands in 2010 was analyzed. These traps caught 2112 individuals of seven amphibian taxa (70 *L. vulgaris*, 757 *T. dobrogicus*, 1151 *B. bombina*, 117 *P. fuscus*, 4 *B. bufo*, 7 *H. arborea*, and 6 *Pelophylax* water frogs).

The number of amphibians and species richness did not differ between restored and natural grasslands. Only the number of *T. dobrogicus* individuals was negatively influenced significantly by ‘Elevation’ ($\chi^2 = 5.4$, $p = 0.02$, **Fig. 6**), indicating more newts in lower-lying areas. None of other dependent variables were affected by any of the factors used in the models.

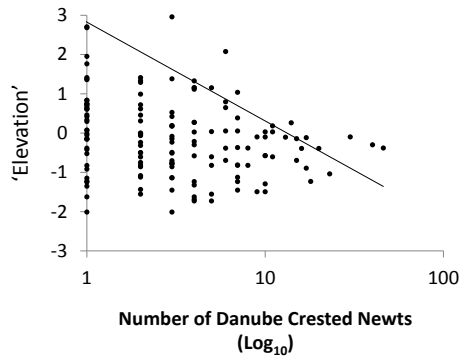


Figure 6. Correlation between *T. dobrogicus* abundance and ‘Elevation’.



5.3. Discussion

This study provides three key findings. First, analyses based on repeated measures showed evidence that several aspects of grassland restoration such as restoration age, last crop and seed mixture used can affect amphibian assemblages. In general, younger restorations hosted higher abundance and richness of amphibians than older restorations. Specifically, the numbers of *B. bombina*, the species richness and the abundance of amphibians were higher in younger restorations (3-6 years) than in older restorations (7-10 years), probably because the vegetation cover of young restorations was diverse providing better hiding and feeding opportunities during the hot summers. Grasslands restored on former sunflower fields had higher species richness and abundance, and more individuals of three species (*L. vulgaris*, *T. dobrogicus*, *B. bombina*) than those restored on former alfalfa fields. Seed mixture affected abundance because there were more individuals in loess than in alkali restorations, although the opposite was found for one species (*L. vulgaris*). Second, chronosequence analyses showed that restoration affected amphibian species both in the short and long term. However, the short-term effects of restoration can differ from those of long-term effects. The studied aspects of restoration did not affect abundance or species richness either in the short or the long term. Restoration age and last crop influenced the number of *B. bombina* individuals in the short term, whereas seed mixture influenced the number of *P. fuscus* individuals. Third, restored grasslands did not differ from natural grasslands in amphibian abundance and richness in the year with the wettest weather and highest total abundance of amphibians (Mester et al. 2017).

Repeated measures suggested that younger restorations can be more suitable for amphibians than older restorations, while chronosequences indicated higher number of *B. bombina* in older (five-year-old)



restorations than in younger ones (2-4 years). This may be explained by more diverse and higher vegetation cover found in 3 to 6 year-old restorations than in older ones (Lengyel et al. 2012). According to Larson (2014) reported that vegetation height is important for amphibians during their seasonal movements as higher vegetation provides better hiding places from avian predators known to prey amphibians, such as the Red-footed Falcon and the White Stork (*Ciconia ciconia* L. 1758) that are frequent in the EPMS (Nagy & Lengyel 2008). The higher vegetation cover also maintains a wetter microclimate (Török et al. 2010), which is also crucial for amphibians in their terrestrial stage.

Both repeated measures and chronosequences showed that former sunflower and cereal fields may be more relevant to amphibians than alfalfa fields, especially in case of *B. bombina* in the short term. The cover of annual weeds disappeared faster and the increase of the cover of perennial grasses can be gradual from former alfalfa fields than from former cereal and sunflower croplands (Lengyel et al. 2012; Kelemen 2013), which may explain why restorations starting from sunflower (and cereal) contained more amphibians.

Repeated measures and chronosequences both revealed the importance of the seed mixture used for restorations on amphibian abundance and the number of *P. fuscus* individuals. The importance of loess mixture to amphibians can be explained by three factors. First, restored and natural grasslands on loess soil located at higher elevation from loess plateaus that may function as connections among the large marshes in the EPMS (Aradi et al. 2003). Amphibians which leave the water to disperse to forage in deeper aquatic habitats or in suitable terrestrial refuges (Jehle & Arntzen 2000; Marty et al. 2005; Kovács et al. 2010), can use these loess plateaus as migration corridors among the marshes. Second, loess grasslands usually have taller vegetation than alkali grasslands (Lengyel et al. 2016) providing better hiding places for amphibians. Third, Mérő et al. (2015a) found that loess plateaus covered



by tall grass are preferred by several small mammal species. Thus, their abandoned burrows can also be suitable for amphibians during the post-breeding movements (Jehle & Arntzen 2000) as refuges in which they can hide from predators and avoid desiccation. The results showed that abundance of *L. vulgaris* can be higher in alkali-restorations than in loess-restored grasslands. For the post-breeding newts, especially the small and slow-moving *L. vulgaris* efts, lower lying alkali-restored grasslands near marshes can be more preferable than the more distant arid loess-restored grasslands.

The three most abundant species (*L. vulgaris*, *T. dobrogicus*, *B. bombina*) in our study provided insight into species-level effects of grassland restoration. PCAs related to aquatic habitats had significant effects on the abundance of each species, emphasizing the importance of nearby wetlands for these species, which are more strongly tied to water than the others (Vitt & Caldwell 2014). The results also support the importance of both restored and natural grasslands adjacent to large wetlands by providing suitable terrestrial habitats for amphibians in the non-breeding period.

In general, chronosequences showed different short-term and long-term impacts of grassland restoration on amphibians. Short-term effects seemed to be slightly similar to what we found with using repeated measures, in the long-term effects. Only the number of *P. fuscus* individuals was affected by seed mixture. This suggests that the loose soil preferring *P. fuscus* (Eggert 2002; Vitt & Caldwell 2014; Glandt 2015), had better conditions to dig burrows in grasslands restored on loess soil than in those restored on alkali soil. *Pelobates fuscus* is known to use mammal-made burrows (Vitt & Caldwell 2014), and it is possible that the higher abundance of *P. fuscus* in loess restorations may be related to the higher abundance of small mammals in loess-restored than in alkali-restored grasslands (Mérő et al. 2015a). The lack of difference in amphibian richness and abundance between restored and natural



grasslands implies that restored and natural grasslands may play similar roles as suitable terrestrial habitat for amphibians during their pre- and post-breeding movements among the wetlands (Jehle & Arntzen 2000; Smith & Green 2005; Searcy et al. 2013). However, Balas et al. (2012) reported that wetlands surrounded by restored grasslands are less frequently occupied by amphibians than those surrounded by natural grasslands (Balas et al. 2012; and Mushet et al. 2014).

This study suggests that grassland restorations can be of importance at two spatial scales. At the local scale, restored grasslands may contribute to maintain hydrological supply because restorations provide the water catchment area of the marshes. If so, the hydro-period of local marshes may be extended, that can benefit several amphibian species (Semlitsch 2000; Baldwin et al. 2006; Hamer et al. 2016). Our results are in accordance with those found by Balas et al. (2012) in the Prairie Pothole Region, in a wetland-grassland complex, where previous drainage for agricultural purposes caused the almost complete extinction of local amphibian populations (Lannoo et al. 1994). Balas et al. (2012) concluded that during arid weather, grassland restorations have an important role in maintaining suitable breeding sites by extending their hydroperiods.

At the landscape scale, grassland restoration may benefit amphibians by increasing the spatial extent of grasslands and ensures connectivity between marshes. If one considers the average spatial scale of an amphibian metapopulation (Smith & Green 2005; Zamudio & Wieczorek 2007; Semlitsch 2008) and the area of the EPMS with several large and numerous smaller marshes in which amphibians are abundant (Mester et al. 2015a; Mester et al. 2017), then the study site can be considered as a possible metapopulation unit in the Hortobágy region for several species. Grassland restorations may thus establish corridors that ensure and maintain connectivity among the amphibian populations in the EPMS. North-to-south-running marshes (e.g. Kis-Jusztus marsh, see **Figure 1**), in turn, can maintain connectivity with other closely located



metapopulations to the EPMS. Thus, genetic erosion of populations induced by isolation can be minimized by grassland restorations.

As a final methodological note, while the repeated measures approach provided detailed information on how various aspects of grassland restoration affected amphibians, chronosequences provided fewer, but also important results. Although using only chronosequences is often the only option available, it can also be effective to measure restoration effects if enough data are available. However, the effect of years cannot be excluded from chronosequences, causing a loss of subtle details (Rácz et al. 2013). As an example for such year effects, the lack of significance for either aspect of restoration based on data from 2010 may be related to the exceptionally wet weather and superabundance of amphibians practically everywhere in the EPMS. Due to the arid weather and the decreased number of traps in 2015, much fewer amphibians were caught in 2015 than in 2010, resulting in lower statistical power and weaker patterns in chronosequence models. Our results thus underline the importance of using repeated measures instead of chronosequences as much as possible to measure the long-term effects of grassland restoration on amphibians.

In conclusion, large-scale grassland restorations have positive impacts on amphibians in a lowland marsh and grassland complex. Increasing the spatial extent of grasslands around and among lowland marshes is an effective tool for conservation that can protect amphibians at both local and landscape scale by ensuring the connection among wetlands and providing terrestrial habitats for foraging and burrowing during the non-breeding period. Furthermore, the results suggest that croplands extending in the water catchment area of marshes or between marshes should be restored to provide connectivity and longer hydroperiods in marshes, which are essential for amphibians in dynamically changing landscapes.



6. Chapter 3 - Compensating Habitat Degradation: The Effects of Conservation Management on Anurans

6.1. Introduction

The most significant tasks of conservation is to ensure landscape-scale diversity to obtain heterogeneous plant and animal communities (Christensen 1997). Moreover, an adequate plan should simulate natural disturbance regimes because these enable the near-natural ecological processes and maintenance of ecological integrity in dynamic landscapes (Mori 2011). If habitat management is considered as an ecological disturbance then it can be characterised for example by its spatial extent, temporal frequency and intensity (White et al. 1999). When this disturbance is at intermediate levels in terms of these characters species diversity can be maximised (intermediate disturbance hypothesis, IDH, Connell 1978). Despite the importance of the IDH in conservation (e.g. Schwilk et al. 1997; McCabe & Gotelli 2000), there is still a knowledge gap, thus it is important to study the optimal intensity and spatial extent of habitat managements.

In case of low levels of disturbances to historically occurring natural disturbances species diversity can decrease due to biotic homogenisation (Lockwood & McKinney 2001). For example, when reed cutting is the only management and is rarely applied in a wetland, the vegetation cover often become homogeneous due to the intense rejuvenation of *P. australis* (Lougheed et al. 2008). If management mimic natural disturbances by changing both spatially and temporally it may break up habitat homogeneity, resulting diverse habitat structure and a wide range of microhabitats accessible for a lot of species (Christensen 1997; Wiens 1997). For instance, spatiotemporally variable management by burning and grazing leads to heterogeneous habitats in both wetlands



(Mérő et al. 2015a) and grasslands (Vinton et al. 1993; Hartnett et al. 1996; Fuhlendorf & Engle 2001).

The decline of amphibian populations presents a global task in conservation (Stuart et al. 2004; Nyström et al. 2007; Pittman et al. 2014). Degradation and pollution of freshwater wetlands are one of the main factors of the decline (Van Den Bos & Bakker 1990; Dodd & Smith 2003; Cushman 2006). Thus, protection and proper management of the aquatic habitats have become an urgent task in amphibian conservation (Schweiger et al. 2002; Bobbink et al. 2006; Smith & Sutherland 2014). Despite the increasing attention to the effects of conservation actions on freshwater habitats (Wagner et al. 2008), there is a knowledge gap in the spatiotemporal allocation of treatments (Ausden et al. 2005; Perry et al. 2012) and its effects on amphibians (Smith & Sutherland 2014). The most frequently implemented conservation actions for amphibians are pond creation, hydrological management of wetlands, and vegetation management in aquatic habitats (Bisson et al. 2003; Pilliod et al. 2003; Hazell et al. 2004; Smith & Sutherland 2014). The latter includes cattle grazing and prescribed fire regimes.

Because of the complexity of species-specific effects which depend on the local habitat structure and amphibian assemblages there is a contradiction in how do prescribed and natural fires impact amphibian assemblages (Hossack & Corn 2007). In the short-term, aridification, increased surface temperatures, UV-B radiation, and predation reported to negatively affect amphibians (Pilliod et al. 2003). In contrast, Russell et al. (1999) and Perry et al. (2012) reported minor negative impacts on amphibians in the mid- and long-term. However, amphibian species occurring in aquatic habitats with a long fire history may have physiological and behavioural adaptations which provide them to survive wildfires. For example, aquatic microhabitats or underground burrows can be optimal refuges for amphibians during a fire (Russell et al. 1999; Pilliod et al. 2003; Roznik & Johnson 2007). From the occurring



amphibian genuses in the Hortobágy region (Dely 1981), Pelobatids and Bufonids may have an evolutionary adaptation to survive fire by burrowing (Nomura et al. 2009), whereas Hylids are reported to be able to avoid wildfires by quickly detecting its sound (Grafe et al. 2002).

Similarly to the fires cattle grazing is also known to have negative effects on amphibians (Jansen & Healey 2003; Burton et al. 2009; Hoverman et al. 2012; Smith & Sutherland 2014). However, most of the studies carried out in aquatic habitats were implemented in forests or grasslands (Larson 2014; Tubbs 2015; Klaus & Noss 2016; Pelinson et al. 2016), thus the combined or separate impacts of wildfires, prescribed burnings and livestock grazing on amphibians in reedbeds or marshes are little known (Smith & Sutherland 2014). The combined impacts of fire and cattle grazing have been also studied only in grasslands (Cano & Leynaud 2009; Larson 2014).

Therefore, this chapter studied the effects of spatiotemporally variable managements on marsh vegetation structure and local anuran assemblages. Prescribed fires and cattle grazing were used to reconstruct the former diversity of the study site that had become homogeneously rejuvenated by *P. australis* due to reed harvesting and constant water supply in the past.



6.2. Results

In total 537 individuals of six frog species (359 *B. bombina*, 118 *Pelophylax* spp., 39 *B. bufo*, and 21 *B. viridis*) were observed in 2010. In total 630 individuals of five species (329 *B. bombina*, 290 *Pelophylax* spp., and 11 *H. arborea*) were observed in 2011. Bufonids were recorded only in 2010 and only in areas burned in 2009 and areas burned in 2007 and 2009, whereas *H. arborea* were found only in 2011 and in areas grazed and burned in 2009 ($n = 7$ frogs) and in three other treatments (in total four *H. arborea* specimen).

6.2.1. Effects of management on reed properties

Mean reed cover was influenced positively by year, increasing from 2010 to 2011 and was influenced negatively by cattle grazing in 2011 as it remained low only in grazed-only areas (**Fig. 7A, Table 6**). However, in 2010 mean reed cover remained also low in transects burned in 2009, whereas it was high in areas burned in 2007 or unmanaged (**Fig. 7A**).

CV reed cover was positively affected by prescribed fire in 2009, and was higher areas burned in 2009 (0.61 ± 0.105 (S.E.), $n = 20$ transects) than in unburned transects (0.31 ± 0.050 , $n = 40$; $t_{27.93} = 2.566$, $p = 0.016$; **Fig. 7B, Table 6**).

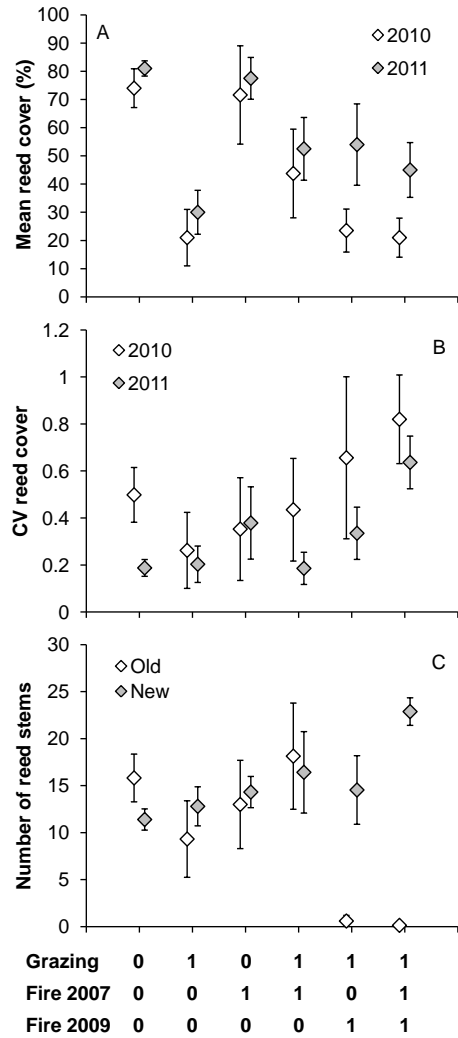


Figure 7. Mean and CV reed cover in two years (A-B), and the number of old and new *P. australis* stems per 0.126 m² in 2010 (C) in transects with different management (means \pm S.E.s shown from $n = 5$ transects for each datapoint; 0: unmanaged, 1: managed). See the results of GLMM models in **Table 6**. The intensity of management combinations are in an increasing order from the left to the right on the x-axis.



Table 6. Generalised linear mixed-effects MaM models testing the effects of management on four variables describing reed properties.

Response variable	Effect	Coefficient	S.E.	df	t	p
Mean reed cover (both years)	(Intercept)	1.03	0.082	29	12.545	< 0.0001
	Grazing	-0.49	0.090	28	-5.454	< 0.0001
	Year	0.16	0.070	29	2.330	0.027
CV reed cover (both years)	(Intercept)	0.31	0.059	30	5.286	< 0.0001
	Fire 2009	0.30	0.103	28	2.916	0.007
Number of old reed stems (2010)	(Intercept)	14.07	1.776	28	7.924	< 0.0001
	Fire 2009	-13.70	3.075	28	-4.455	< 0.0001
Number of new reed stems (2010)	(Intercept)	12.91	1.599	28	8.074	< 0.0001
	Fire 2007	4.96	2.262	28	2.193	0.037



The before-after comparison study resulted a significant decrease in mean reed cover from $55.6 \pm 37.71\%$ (S.D., in 2007, before the fire) to $35.7 \pm 22.58\%$ in 2008 (Wilcoxon's test, $z = 3.617$, $n = 30$, $p < 0.001$). In contrast, ten plant species were detected before and 18 species after the fire. Nine plant taxa appeared (*Atriplex hastata*, *Bidens tripartitus*, *Chenopodium chenopodioides*, *Galium palustris*, *Inula britannica*, *Persicaria* spp., *Rumex stenophyllus*, *Schoenoplectus lacustris* and *Sonchus arvensis*) and one species (*Salix caprea*) disappeared. The species richness of plants increased from 2.9 ± 1.46 before to 4.0 ± 2.4 after the fire ($z = 3.264$, $n = 30$, $p = 0.001$). However, three species (*Epilobium tetragonum*, *Lycopus europaeus* and *Rumex palustris*) increased their cover after the prescribed fire.

The old reed stems in areas burned in 2009 were almost completely removed to 2010 (mean number of stems; 0.4 ± 0.3 stems per 0.126 m^2 , or c. $3.2 \text{ stems} \cdot \text{m}^{-2}$, $n = 10$ transects), whereas the number of old reed stems was significantly higher ($t_{19,73} = 6.309$, $p < 0.0001$; **Fig. 7C**) in other areas (mean number of stems; 14.1 ± 2.15 or c. $113 \text{ stems} \cdot \text{m}^{-2}$, $n = 20$), indicating a significant negative impact of prescribed burning of reed in 2007 on the number of old reed stems (**Table 6**). In addition, the number of old reed stems in transects burned in 2007 was comparable to control or grazed-only areas (**Fig. 7C**).

The new reed stems in areas burned in 2009 were regrown in 2010, whereas the number of new and old reed stems did not differ from each other in areas burned in 2007 (**Fig. 7C**). However, prescribed fire in 2007 positively affected the number of new reed stems (**Table 6**), because the number of new reed stems in 2010 was significantly higher in areas burned in 2007 (17.9 ± 1.78 , $n = 15$) than in other areas (12.9 ± 1.38 , $n = 15$; $t_{26,34} = 2.193$, $p = 0.037$; **Table 6**). Finally, the rejuvenation of reed was dynamic in areas burned bot in 2007 and 2009 (**Fig. 7C**).



6.2.2. Effects of reed properties on amphibians

Only mean reed cover affected significantly species richness, when data from both 2010 and 2011 were analyzed (**Table 7**), and it had a negative impact, indicating fewer species in areas with more dense reed cover (**Fig. 8A**).

Total abundance of amphibians was affected by significant interactions between year and mean reed cover and between year and CV reed cover (**Table 7**). The reason of the former interaction was that total abundance of amphibians decreased faster with mean reed cover in 2011 than in 2010 (**Fig. 8B**). The reason of the latter interaction was that total abundance of amphibians increased with CV reed cover in 2010 but not in 2011, although the range of CV reed cover in 2011 was also broadly half of what it was in 2010, indicating more homogeneous cover by *P. australis* (**Fig. 8C**).

In the next year after the last fire management, the number of old reed stems had negative impacts on both anuran richness and abundance (**Table 7, Fig. 8D-E**). In the next spring after the prescribed fire in 2009, two Bufonids were observed only in areas burned in 2009 (*B. bufo*; n = 39) or in areas burned in 2007, and areas burned in 2009 and ungrazed (*B. viridis*; n = 21). Total abundance of amphibians was positively affected by the number of new reed stems (**Table 7, Fig. 8F**), because the cover of newly regrown reed was high in areas burned in 2009 (**Fig. 7C**) in which anurans were numerous.

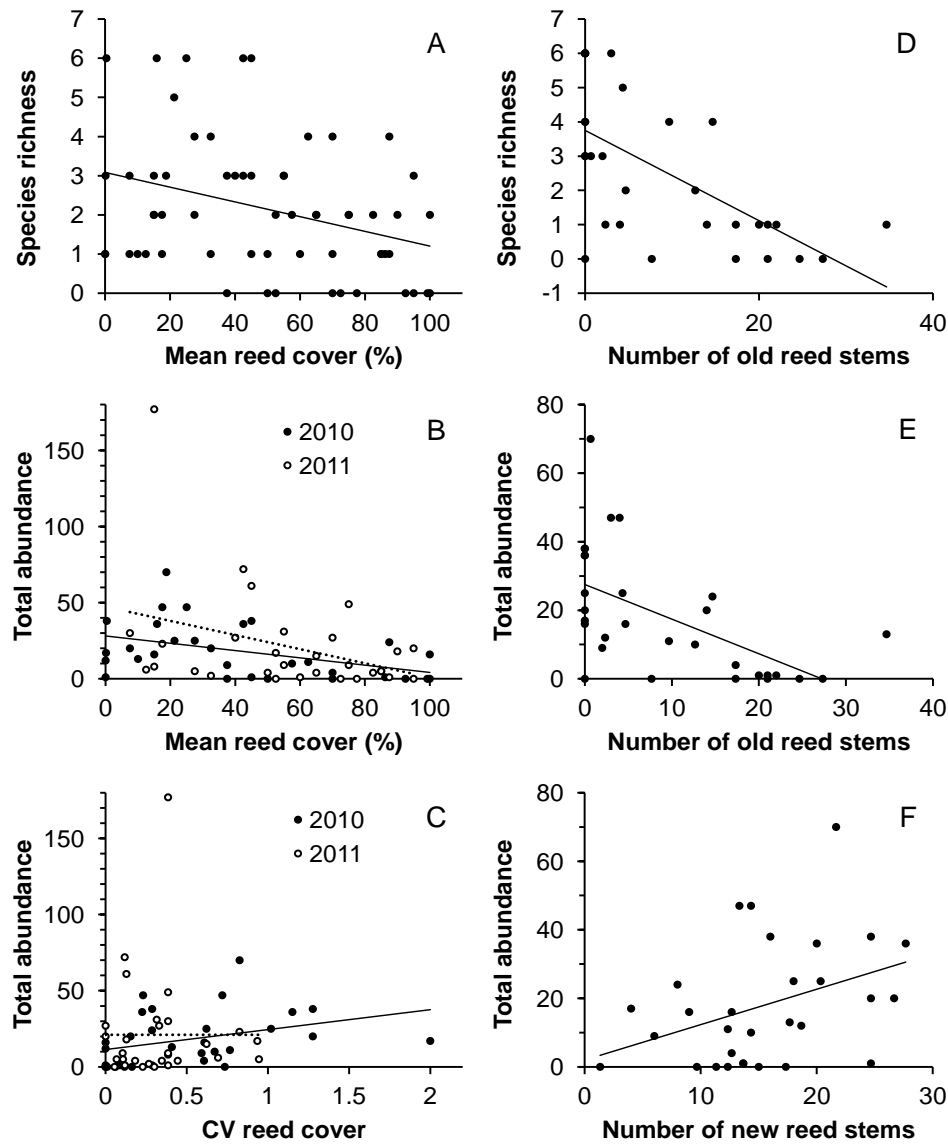


Figure 8. Anuran richness and abundance as a function of mean and CV of reed cover (both years, A-C), and number of old and new reed stems (2010 only, D-F) per transect. Least-squares regression lines are for visual guidance only; see statistics such as parameter estimates from GLMM models in **Table 7**.



Table 7. GLMM MaM models testing the effects of reed properties and environmental variables on species richness and number of anurans in both years and in 2010 only, when more detailed data on reed density were available.

Response variable	Effect	Coefficient	S.E.	z	p
Species richness (both years)	(Intercept)	1.25	0.18	6.924	< 0.0001
	Mean reed cover	-0.66	0.231	-2.863	0.004
Total abundance (both years)	(Intercept)	2.74	0.354	7.737	< 0.0001
	Mean reed cover	-1.31	0.278	-4.725	< 0.0001
	Year	3.85	0.323	11.922	< 0.0001
	CV reed cover	0.32	0.204	1.545	0.122
	Cloud cover	0.30	0.028	10.673	< 0.0001
	Mean reed cover*Year	-2.90	0.305	-9.516	< 0.0001
	CV reed cover*Year	-4.36	0.372	-11.718	< 0.0001
Species richness (2010 only)	(Intercept)	1.39	0.148	9.387	< 0.0001
	Number of old reed stems	-0.07	0.017	-4.278	< 0.0001
Total abundance (2010 only)	(Intercept)	1.29	0.698	1.847	0.065
	CV reed cover	0.99	0.464	2.137	0.033
	Number of old reed stems	-0.09	0.024	-3.611	< 0.001
	Number of new reed stems	0.07	0.033	2.099	0.036



6.2.3. Effects of management on amphibians

The results on how do managements affect frogs showed that anuran richness was affected by significant interactions between year and grazing and between year and burning in 2009 (**Table 8**). The reason of the former interaction was that the number of anuran species increased from 2010 to 2011 in grazed-only areas and in areas burned in 2007 and grazed, but decreased in unmanaged transects and in areas burned in 2009 (**Fig. 9A**). The reason of the latter one was that the number of species in 2010 was higher in areas burned in 2009 than in other areas but not in 2011 (**Fig. 9A**). Species richness was also positively affected by cloud cover (**Table 8**). Finally, total abundance was also affected negatively by water depth and positively by cloud cover and wind speed (**Table 8**).

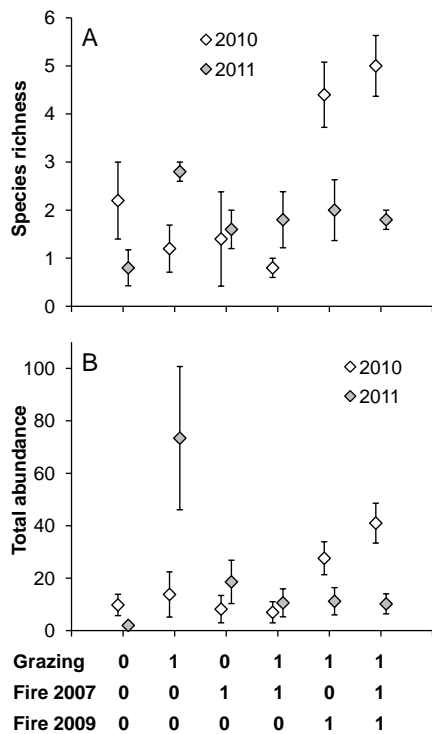


Figure 9. Mean \pm S.E. anuran richness and abundance in transects with different treatments ($n = 5$ transects for each datapoint; 0: unmanaged, 1: managed). The intensity of management combinations are in an increasing order from the left to the right on the x-axis.



Table 8. GLMM MaM models testing the impacts of cattle grazing, year, prescribed fire in 2007 and 2009, and environmental variables on anuran richness and abundance.

Response variable	Effect	Coefficient	S.E.	z	p
Species richness	(Intercept)	0.72	0.259	2.759	0.006
	Grazing	-0.71	0.396	-1.784	0.074
	Year	-0.61	0.401	-1.518	0.129
	Fire2009	1.81	0.369	4.896	< 0.001
	Water depth	-0.02	0.009	-1.952	0.051
	Cloud cover	0.13	0.050	2.591	0.010
	Grazing*Year	1.48	0.538	2.757	0.006
	Fire2009*Year	-1.94	0.492	-3.949	< 0.0001
Total abundance	(Intercept)	1.86	0.358	5.198	0.000
	Grazing	-0.74	0.483	-1.532	0.126
	Year	-0.60	0.195	-3.094	0.002
	Fire2009	2.64	0.481	5.482	< 0.001
	Water depth	-0.05	0.006	-7.554	< 0.001
	Wind speed	0.24	0.066	3.673	< 0.001
	Cloud cover	0.39	0.037	10.397	< 0.0001
	Grazing*Year	2.23	0.230	9.673	< 0.0001
	Fire2009*Year	-3.40	0.208	-16.334	< 0.0001

Anuran abundance was affected by significant interactions between year and cattle grazing and between year and recent burning (**Table 8**). The reason of the former interaction was that total abundance increased in grazed-only areas from 13.8 ± 8.61 frogs (range: 0-47) in 2010 to 73.4 ± 27.32 frogs (range: 27-177) in 2011, whereas it did not change much in ungrazed transects (**Fig. 9B**). The increase in abundance in grazed-only transects was resulted both for the *Pelophylax* spp. individuals and *B. bombina* (**Fig. 10**). The reason of the latter interaction was that total abundance decreased by more than half from 2010 to 2011 in areas burned in 2009 (**Fig. 9B**), likely due to the rejuvenation of *P. australis*.



The decrease in these areas was higher for *B. bombina* than for *Pelophylax* spp. (**Fig. 10**).

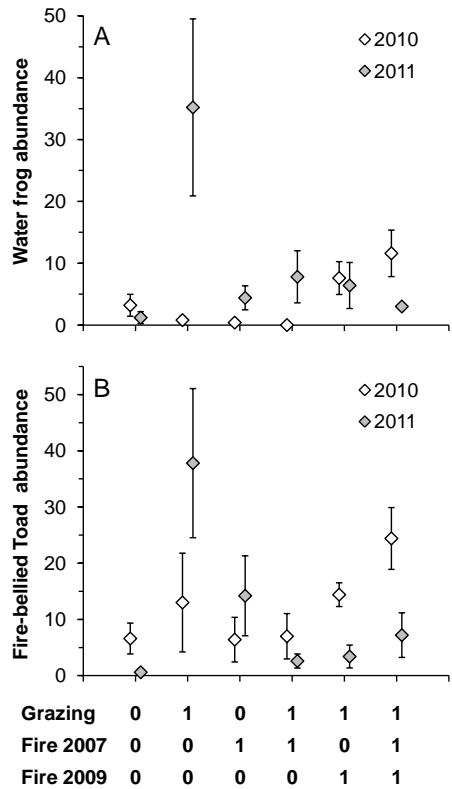


Figure 10. Mean \pm S.E. abundance of (A) *Pelophylax* spp. and (B) *B. bombina* in transects with different treatments ($n = 5$ transects for each datapoint; 0: unmanaged, 1: managed). The intensity of management combinations are in an increasing order from the left to the right on the x-axis.



6.3. Discussion

The results of this chapter showed that marsh management by low-intensity grazing and prescribed fire can effectively open up homogeneous reedbeds and increase the plant and anuran diversity.

Prescribed fire carried out in late-summer almost completely erased old reed stems, reduced reed cover in general, increased the variability in reed cover, and also increased the species richness and diversity of the marsh vegetation the next spring. However, the prescribed fire itself eliminated the cover of *P. australis* for a very short time period because it grew back fast from rhizomes the next year, indicating that fire enables the quick rejuvenation of reed (Valkama et al. 2008). Beside this mean reed cover in 2010 and 2011 was either high or intermediate in old burned areas and it decreased back to intermediate levels by 2011 in recent burned areas. In addition, the number of new reed stems was positively related to prescribed fire in 2007 and increased rejuvenation was recorded in areas burned twice. These findings imply that the disturbance effect of fires vanishes very shortly (2-3 years). Thus, it allows only short-term benefits to the marsh vegetation and therefore to frog species.

Conversely, in grazed transects both mean and CV reed cover remained low. Grazing and trampling by cattle effectively controlled the cover of *P. australis*, thus increased habitat diversity and maintained microhabitats with diverse plant communities and open water surfaces preferred by frogs in spring. This finding was supported by (i) more anuran species and *Pelophylax* spp. abundance and more Bufonids in areas burned the previous summer, (ii) the negative correlations between species richness or frog abundance and mean reed cover or the number of old reed stems, and (iii) the positive correlations between total abundance of anurans and CV reed cover. One possible reason why was the abundance of frogs higher in recent burned areas is that the vegetation cover was very low in these areas, thus the water temperature could arise



quicker compared to areas with dense and old reedbeds. Shallow and warm waters with better exposure to the sun due to the low vegetation cover may be beneficial to thermoregulating frogs, in particular on the cold days of spring (Puky et al. 2005; Vitt & Caldwell 2014). Quicker warming results in a greater time window to foraging and mating. Thus, microhabitats providing small, open water patches which is likely a key factor for the local frog assemblage. To sum up these results, spatial and temporal changes in reed cover due to prescribed fire and cattle grazing used together benefitted anurans.

Direct connection between species richness and cattle grazing was also found. Anuran richness increased from 2010 to 2011 only in areas burned in 2007 and grazed-only areas, but decreased in recent burned and control areas. Similarly, in 2010, total abundance of frogs were highest in areas burned in 2009, whereas in 2011, total abundance were highest in grazed-only areas, and a large decrease in recent burned and control areas was found. These findings highlights that reed burning and cattle grazing have different effects on anurans (see **Appendix 5**).

Because grazing by cattle is likely to keep mean reed cover low along an entire transect, and mean reed cover was associated with higher anuran richness and abundance, cattle grazing may have a more essential impact on reed compared to prescribed or wildfires. One interpretation can be that livestock not only consume the reed during a whole vegetation period but also damage the rhizomes by the trampling (Hamer & McDonnell 2008). However, its effectiveness in the eradication of reed likely depends on the stocking rate and grazing regime applied (Jansen & Healey 2003). Our results imply that over longer terms (> 4 years), even low grazing pressure can keep the reed cover low. Taken together, the findings of this study support the theory that marsh management combining prescribed fire and cattle grazing can create various habitat patches, some of which will be optimal in one year, while others



becoming suitable in a subsequent year when successional changes modify the previously optimal habitat patches (**Appendix 5**).

Finally, these results also show that management intensity influenced anuran richness and abundance (see **Figs. 7, 9 and 10**). This is because the highest anuran richness and abundance were found for high-intensity treatments in 2010 and for a low-intensity management in 2011. Intermediate levels of management intensity, for example only burned in 2007 or grazed and burned in 2007, did not have more species or individuals than either high- or low-intensity treatments. Therefore, the results do not support the IDH.

Based on the available literature, this chapter firstly reports both direct and indirect effects of fire and grazing management on anurans in freshwater wetlands, thus it can reduce the knowledge gap in wetland conservation (Valkama et al. 2008). A similar survey on the local bird assemblages also highlights that the applied conservation actions resulted in higher diversity of habitats also increasing the diversity of birds (Mérő et al. 2015b). The result of this chapter also agree with those of Perry et al. (2012), who found more toads after a fire implemented in forests, and suggests that prescribed fires can also benefit toads in wetlands. In contrary, a similar study carried out in grasslands reported that prescribed fire combined with intensive cattle grazing had negative effects on amphibians (Cano & Leynaud 2009). These difference can be explained by that fires have more negative impacts on amphibians in dry habitats such as grasslands compared to wetlands, and in the present study low intensity grazing was conducted in an aquatic habitat compared to high intensity grazing in a grassland.

Despite the benefits of cattle grazing in marshes, previous literatures showed lower amphibian richness, abundance and diversity in grazed wetlands, due to direct mortality from trampling, and indirect mortality from increased predation, solar and UV-B radiation, desiccation, and eutrophization, or the presence of *Ranavirus* (Jansen &



Healey 2003; Schmutzer et al. 2008; Burton et al. 2009; Hoverman et al. 2012). Because of the low level of grazing intensity and the spatiotemporal manner of marsh management applied in this study these factors may not cause direct or indirect mortality of local anurans.

To conclude the results of this chapter, low-intensity cattle grazing and late-summer prescribed burning of reed can create and maintain diverse habitats preferred by post-metamorphic frogs. Low-intensity grazing keeps the cover of reed low and has little negative impact on anurans, but it becomes beneficial over longer time periods. In contrary, late-summer prescribed fire effectively eradicates reed. However, it is essential to be repeated once every 2-3 years to benefit anurans due to its rapidly vanishing effect caused by the quick regrowth of reed. Thus, the results highlight the view that marsh management varying in space and time can lead to a mosaic vegetation and habitat structure that benefits frogs. The results also support previous calls for spatiotemporal management to facilitate habitat diversity and complexity in dynamically changing landscapes (Christensen 1997; Parr & Andersen 2006).



7. Chapter 4 - Compensating Habitat Fragmentation: Local Amphibian Migration, Road Mortality and Current Mitigation Measures

7.1. Introduction

Negative effects of linear structures - in particular roads - on wildlife have been in the spotlight since the last decades of the 20th century (Colino-Rabanal & Lizana 2012). Increasing traffic density and intensity have indirect and direct effects on wildlife (Garrah et al. 2015). Several field studies and reviews reported indirect effects of habitat fragmentation by linear structures, which leads to the degradation and loss of natural habitats (Wu 2013; Ibisch et al. 2016; Wilson et al. 2016). Habitat and landscape level changes influence the genetical diversity, abundance, dispersal and migration habits and distribution of animal populations (Gunson et al. 2011). Direct mortality by traffic (road kill) became the major part of the studies on the negative effects of linear structures as millions of vertebrates are killed every day by vehicles (Woltz et al. 2008). Collision with larger animals also raises safety issues as it can lead to human injury or fatality (Gunson et al. 2011).

Amphibians are the vertebrate taxa with the highest road mortality due to their migration and dispersal habits among wetlands and between aquatic and terrestrial habitats which are isolated by roads (Hels & Buchwald 2001; Garrah et al. 2015; Garriga et al. 2017). The probability of mortality by vehicles are high for amphibians which are slow-moving, have small body size and often use the road surface for thermoregulation (Hels & Buchwald 2001; Langen et al. 2009).

To decrease the impact of road kill, more than 2000 mitigation measures have been carried out around the world in at least 20 countries, mostly in Europe and North-America, since 1969 (Ryser & Grossenbacher 1989; Puky 2003; Jochimsen et al. 2004). However, the number of amphibian road crossing sites is probably several times higher



than the number of existing mitigation measures, and the effectiveness of many mitigation measures in reducing amphibian road mortality is poorly understood (Puky 2003). First, earlier studies localised road kill hot spots based on the counted number of dead amphibians (see e.g. Garrah et al. 2015). However, this method can be biased because old roads with low traffic intensity, even with low quality surrounding habitats, can easily become a hot spot due to population depression in neighboring areas caused by road mortality due to higher traffic intensity (Teixeira et al. 2017). Second, an understanding of the effectiveness of mitigation measures by meta-analytical methods is limited by the low number of published studies (Gunson et al. 2011; Rytwinski et al. 2016). Thus, ineffective mitigation measures can still lead to possible extinction of local amphibian populations in only a few generations. For example, population models predicted a survival of only 20 to 40 years for a population of Northern Red-legged Frogs (*Rana aurora* Baird and Girard, 1852) at an ineffective passageway system along the Sea to Sky Highway in western Canada (ECCC 2017).

In Hungary, the first system of fences and modified culverts (drainage pipes below the road) was constructed at Parassapuszta in 1986 (Csincsa 1986), but it was ineffective due to the low angle of the concrete fence (Puky 2003). Several mitigation measures have been implemented later in the country, for example in the HNP, where the first fence and tunnel system was made in the 1990s (Puky 2003). This system was renewed and extended later in the early 2000s, but later it became almost completely abandoned. In 2012 a new and much longer (in total 23 km) plastic fence system was placed along the Hortobágy section of the Main Road 33 (see **Appendix 6**) to mitigate road mortality of amphibians and other vertebrate taxa). However, amphibian road mortality data were not available from this area despite the high effort and costs of building the systems and the protected status of the area.



7.2. Results

In total 16,441 individuals of eight amphibian taxa (*L. vulgaris*, *T. dobrogicus*, *B. bombina*, *P. fuscus*, *B. bufo*, *B. viridis*, *H. arborea*, *Pelophylax* water frogs) were observed. The most common species was *P. fuscus*. *H. arborea*, *B. bombina*, *T. dobrogicus* and *L. vulgaris* were also common in road sections close to wetlands. *B. viridis* was numerous along the residential areas, whereas *B. bufo* mainly occurred along the EPMS, in particular in the area of Hagymás-lapos and Hagymás-fertő marshes. Most of the *Pelophylax* spp. individuals were observed on road sections near fishponds and canals.

Number of roadkilled amphibians were significantly affected by survey year and survey period (early or late spring) and the presence of the EPMS along the sections (**Table 9**). None of the other variables used in the models (e.g. proportion of fence, presence of tunnels and 13 variables describing the proportion of habitat types) affected the number of roadkilled amphibians.

Table 9. A GLME MaM model testing the effects of survey year and period, mitigation measures (plastic fence and tunnels), neighboring landscape structure, and the occurrence of the EPMS on the number of roadkilled amphibians in a repeated measures design using data collected in ten roadkill surveys between 2013 and 2017.

Variables in final model	X ²	p	Relationship/difference
Survey year	189.96	< 0.001	2014>2013>2015=2016=2017
Survey period	17.11	< 0.001	late > early
Presence of the EPMS	31.68	< 0.001	EPMS > non-EPMS



The roadkill of amphibians was the highest in 2014, and the lowest in between 2015 and 2017, with 2013 being an intermediate year in term of amphibian road mortality (**Fig. 11A**). Amphibian roadkill was significantly higher in late spring than in early spring (**Fig. 11B**), and it was significantly higher along the EPMS than elsewhere (**Fig. 11C**).

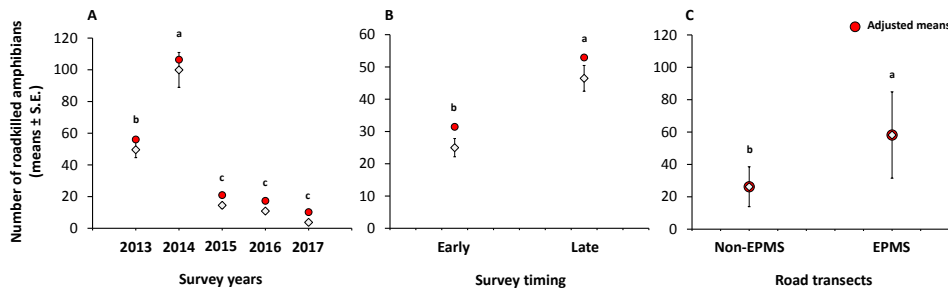


Figure 11. Means and adjusted means of roadkilled amphibians (**A**) among the survey years, (**B**) the survey period, and (**C**) the non-EPMS and EPMS road sections. Different lowercase letters indicate significant differences (Tukey HSD test after GLMM in **Table 9**, $p < 0.05$).

Dytime traffic intensity was on average 2-3 vehicles/minute during the surveys, and decreased to on average 1 vehicle/minutes for evening and then 0.3-0.5 vehicles/minute for nighttime. Daytime, evening and nighttime traffic intensity have shown a decreasing trend since 2006 (**Fig. 12A**).

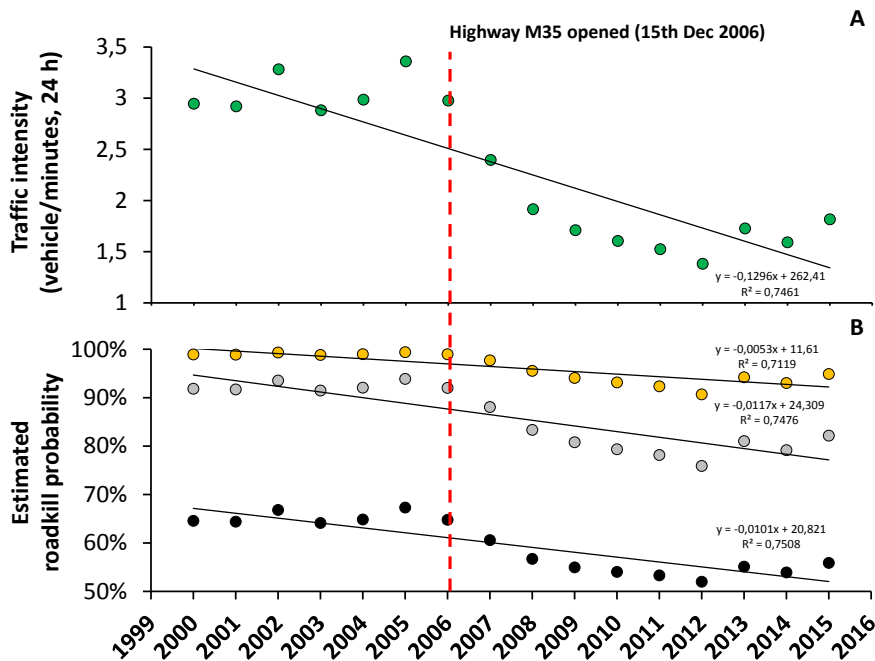


Figure 12. Changes in (A) traffic intensity with a trend line and (B) estimated amphibian roadkill probabilities with trend lines for daytime (6 am to 6 pm), evening (6 pm to 10 pm) and nighttime (10 pm to 6 am) periods analysing long-term (16 years) traffic data (HPR 2017). Dashed line in red highlights the date when Highway M35 was opened for public use.

Traffic intensity of the past 10 years (2006-2015) was on average 39% of the traffic intensity in the previous six years (2000-2005, see **Fig. 12A**). The estimated roadkill probability of amphibians followed the decrease in traffic intensity (**Fig. 12B**). Estimated roadkill probability decreased with 14% for daytime (linear trend, $r^2 = 0.71$), 11% for evening (linear trend, $r^2 = 0.74$), and 5% for nighttime (linear trend, $r^2 = 0.75$) compared to the average of the previous six years (2000-2005, **Fig. 12B**).

Amphibian roadkill probability was estimated between 52% and 99.4% depending on the traffic intensity and the time of the day. This



probability ranged from 90.7% to 99.4% in daytime (from 6 am to 6 pm), from 75.9% to 93.9% in evening (from 6 pm to 10 pm), and from 52% to 67.3% in nighttime (from 10 pm to 6 am) (see **Fig. 12B**). Estimated roadkill probability increased with traffic intensity along a saturation curve (**Fig. 13**). The probability reached 90% at a traffic intensity of only c. 2 cars/minute. However, the rapid growth of roadkill probability slowed down as traffic intensity exceeded the traffic intensity of 2 cars/minute.

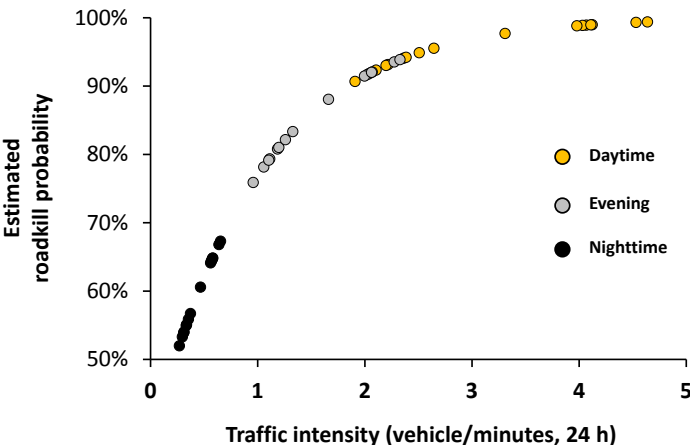


Figure 13. Relationship between estimated roadkill probability of amphibians and traffic intensity in daytime, evening and nighttime using 16 years of survey data (HPR 2017).



7.3. Discussion

The results showed that road mortality of amphibians has both spatial and temporal differences along the studied road section of Main Road 33. The only spatial difference related to the roadside landscape structure was that the number of roadkilled amphibians was higher in the road transects along the EPMS than elsewhere. This result confirms that the EPMS is an important wetland for amphibians at the landscape scale, as are large wetland complexes in general (Langen et al. 2009; Gunson et al. 2011; Garrah et al. 2015; Mester et al. 2017). However, the protected status of these wetlands in itself does not mitigate the road mortality of local amphibians (Garriga et al. 2012). Therefore, even protected areas, especially those physically connected to high-quality roadless areas, such as the EPMS, need effective mitigation measures to decrease the impacts of roads on wildlife. Although road mortality hotspots may not be the best sites for mitigation measures (Teixeira et al. 2017), the entire 47-km section can be considered as a high-traffic road because of the high traffic volume and estimated road mortality probability. My results thus suggest that the EPMS is a potential roadkill hotspot due to the proximity and high proportion of valuable aquatic and terrestrial habitats for amphibians and the high traffic intensity and velocity.

One of the most intriguing results of this study was that neither the plastic fence nor the tunnels did not affect amphibian roadkill. There may be several reasons for this. First, although fencing alone appeared to reduce road mortality in many studies, fencing is not a completely effective mitigation measure due the fence-endings, where animals can cross the road (Rytwinski et al. 2016). Despite its length, the plastic fence along Main Road 33 also suffers from this failure as it is separated into three distinct sections which themselves also are not continuous due to incoming roads, crossing canals and damaged fence sections. Second, several constructional defects weaken the effectiveness of this fence.



Moreover, drainage and wildlife tunnels are not maintained. Third, even in some sections where the fence is still functioning, the fence was built on the farther bank of the roadside drainage ditch, and the fence thus effectively traps the amphibians in the ditches that hold water. Moreover, amphibians with good climbing ability such as *H. arborea* can easily climb over the plastic fence. Thus, numerous individuals can be killed by the traffic as they are forced to move between the fences, crossing the road several times.

The road mortality peak in 2014 can be linked to the high amount of precipitation during the two surveys in 2014 (14.1 mm, sum of 6 days) compared to the other survey years (2013: 5mm, 2015: 3mm, 2016: 7.9mm, sum of 6 days per year) (Wunderground 2017). The spring migration of amphibians is triggered by the first rainfall peaks (Garriga et al. 2017). However, migration peaks also often occur in wet and warm weather in late spring (e.g. April) (Langen et al. 2007; Garriga et al. 2017), in agreement with my findings (**Fig. 11B**). Moreover, (Garrah et al. 2015) reported that road mortality of frogs was higher when the sum of precipitation was above 6.4 mm the previous day. One survey in late March in 2014, when I found high road mortality after a quick and heavy rainfall (total 7.1 mm), also supported this observation.

The temporal variability in road mortality is also affected by the temporal variation in traffic intensity (Garrah et al. 2015). Since Highway M35, which diverted much of the traffic from Main Road 33, has been in use (late 2006), traffic intensity of Main Road 33 decreased considerably in the Hortobágy region. Although amphibian road mortality probability also showed a slightly decreasing trend for the past 10 years, it was still high during the survey years, especially in the daytime and evening when migration and dispersal movement of amphibians is frequent (Semlitsch 2008). However, it has to be noted that my estimates of road mortality probability are valid for individuals crossing the road at right angles (Hels & Buchwald 2001). Thus, road mortality probability for amphibians



which use the road surface for thermoregulation can be higher compared to the estimates for migrating or dispersing individuals, even in nighttime when traffic intensity is the lowest.

Considerable attention has been given to the role of roadless areas in conservation recently (Selva et al. 2011). This is because the size of roadless areas (based on 1-km buffer zones along the roads) in Europe is decreasing and their fragmentation is increasing due to linear structures (Ibisch et al. 2016). Moreover, only a low proportion of high-quality roadless patches is protected (Ibisch et al. 2016), and their edges are exposed to the negative effects of linear structures (Garriga et al. 2012). My study suggests that the EPMS is a good candidate for a high-quality roadless area, which is nevertheless influenced by the negative edge effects arising from Main Road 33 that runs along the entire southern edge of the EPMS.



8. General Conclusions & Implications

Chapter 1 presented fine-scale occurrence data on amphibian species and the frequency and types of amphibian morphological anomalies observed in the EPMS. The dataset expanded the number of amphibian species detected in the two 10×10km UTM cells covering the area by five and seven new species, respectively. Large populations of two species (*T. dobrogicus* and *B. bombina*) listed in Annex II of the EC Habitats Directive were found in the study site. Chapter 1 also showed that malformations were absent and the abnormalities observed were consistent with injuries caused by predators, although the effects of parasites could not be excluded. Chapter 1 also presented the first evidence of tail bifurcation in *T. dobrogicus*. However, it remained uncertain whether the absence of malformations and the observed low frequency of abnormalities were related to the 40 years of protection and the long-term decrease in agrochemical use or to the more recent grassland restoration and marsh management actions. Nevertheless, this study provided an example that large, healthy populations of amphibians can exist in large protected wetland complexes protected, restored and managed for biodiversity conservation purposes.

One very important implication of Chapter 1 was that datasets on the occurrence, abundance and condition of amphibian populations should be collected, integrated, synthesized and published for use in the design and implementation of practical conservation actions. It is also necessary to interpret the results for decision makers, because publishing only the raw data would not save the natural heritage (Ellison 2016). For instance, in Hungary, there are several parallel running monitoring programmes organized by the government, national parks, NGOs, scientists, conservation professionals and even civilians, but the availability and quality of these data on amphibians are highly variable. Integrating these data and making them open-access could significantly improve our knowledge on the recent distribution and trends of amphibian populations



in Hungary and Europe. The use of novel methods such as machine learning for analysis and interpretation could give further weight to our messages by presenting the recent status and the expectations under future scenarios, which can be demonstrative and highly convincing for decision-makers.

Chapter 2 highlighted positive impacts of large-scale grassland restoration on amphibians in a lowland marsh and grassland complex using long-term data (up to 10 years) and provide three main results. First, repeated measures analyses demonstrated that three key aspects of grassland restoration (restoration age, crop history and seed mixture used) affected amphibians. In general, early phases of restoration, that were dominated by weedy and dicotyledonous plant species, provided good hiding places and wetter microclimate to amphibians. Later phases, characterised by the dominance of grasses and a fewer number of dicotyledonous target plant species, had a lower availability of microhabitats for amphibians. Finer-scale differences in crop history (more newts in former sunflower fields) and seed mixture (more amphibians in loess restorations) also caused variation in the abundance and richness of amphibians in restored grasslands.

Second, chronosequences also highlighted differences among short- and mid-term effects of restoration (more *B. bombina* in 5-year-old grasslands than in younger ones and more *P. fuscus* in later than in early-phase restorations) providing additional details to what repeated measures found.

Third, the results also showed the importance of grassland restoration in amphibian conservation as restored grasslands did not differ from natural grasslands in the number of individuals of species, total abundance or species richness.

The results of Chapter 2 demonstrated that increasing the spatial extent of grasslands around and among lowland marshes is an effective tool for conservation which can protect amphibians both at the local and the landscape scales by ensuring the connection between the wetlands and



providing suitable terrestrial habitats for foraging, burrowing and overwintering. In addition, the results of Chapter 2 also implied that croplands in the water catchment area of marshes or between wetlands should be restored first to provide connectivity and longer hydroperiods for the marshes which are essential for amphibians in dynamically changing landscapes.

Chapter 3 showed that fire management of homogeneous reedbeds provides short-term benefits to anuran amphibians by increasing the diversity of habitats in the marshes. However, this positive effect disappeared quickly due to the rapid regrowth of reed, indicating that fire management must be applied frequently to old reedbeds to extend the benefits to amphibians. In contrast, low intensity rotational cattle-grazing kept reed cover low and created open water patches favoured by amphibians. Direct evidence was presented that management by fire and cattle-grazing provided benefits to amphibians by modifying the structure of the vegetation and increasing the diversity of habitats. These results suggested that grazing and burning combined can create different habitat patches, some of which will be suitable for amphibians in one year, whereas others may become optimal in a subsequent year. These results call for the application of spatiotemporally varied, mosaic-like management to mimic natural disturbances and to maintain habitat diversity and vegetation complexity in dynamically changing landscapes.

The results of Chapter 3 had three main implications for conservation practice. First, it is recommended to use fire management and cattle-grazing in combination to restore and maintain habitat and species diversity in homogeneous reedbeds as the two are complementary rather than supplementary. The direct negative effects of cattle-grazing to amphibians can be minimised and the indirect, habitat-level benefits to amphibians can be maximised if grazing is conducted at low stocking intensity (likely not much more than the 0.6 livestock unit per hectare applied here) and in a rotational manner. Second, to reap the full benefits of fire management and to minimise direct mortality to amphibians,



controlled fires should be carried out in the late summer at the flowering of the reed plant and in a patch mosaic burning (PMB) arrangement, which is considered more favourable as it leaves suitable refuges to amphibians (Parr & Andersen 2006). In a long-term fire management programme, local fire intervals should be carefully established, particularly if grazing or periodic flooding are also planned (Hackney & de la Cruz 1981; Coops & Hosper 2002; Parr & Andersen 2006; Matthews et al. 2010). Ideally, PMBs should also be implemented in a rotational manner; for example, if the fire interval is set at three years, one-third of the area should be burned every year to maximise the continuity of optimal habitats. Third, these results also highlights that if the major goal of prescribed fire is to destroy reed, the rhizome has to be drowned by flooding the next winter. Otherwise, reed will quickly regrow from the rhizome in areas that were burned (Valkama et al. 2008). Theoretical education and field training courses on prescribed fire management should also be carried out for land managers including national parks to acquire the proper knowledge which ensures high safety and effectiveness.

Chapter 4 found that the road mortality of amphibians varied spatially and temporally along the 47-km road surveyed. Chapter 4 also found that the 14-km section bordering the EPMS can be a possible roadkill hotspot compared to the other sections of the road. It was because of the high traffic intensity, the lack of effective mitigation measures and the large numbers of amphibians in the marshes, canals and natural and restored grasslands close to the road. Neither the plastic fencing nor the drainage or passage tunnels affected amphibian roadkill along the 47-km road because of constructional issues and the lack of maintenance. Estimated roadkill probability was high especially in daytime and the evening due to the weak mitigation measures and the high traffic intensity and could reach 100% for amphibians that (i) use the road surface for thermoregulation, (ii) cross the road at low angles (crossing is longer), (iii) cross the road between two neighboring aquatic habitat or roadside



plastic fence section over and over again. Unfortunately all these cases are present along the studied road. Although traffic intensity has decreased in the past 16 years, it is still high (above 1 car/minute) causing high road mortality.

Chapter 4 also showed that amphibian road mortality varies by year, season and by the time of the day. Inter-year fluctuations may be related to variation in precipitation, with more amphibians killed in wetter years. More road-killed amphibians were found in late spring surveys than in earlier ones, which may be related to the later pre-breeding movement peaks of numerous late migrating amphibian species of the region. Finally, most roadkill occurred during the daylight and evening hours, when traffic is heavier than in the nighttime.

The results of Chapter 4 implied that active measures are necessary to decrease the road mortality of amphibians along the Hortobágy section Main Road 33, especially in areas where it separates large marshes and important terrestrial habitats. First, the best solution is to use ecopassages which are placed along the local roadkill hot spots. Extended stream crossings that are at least five times wider than the canals below the road can be effective with concrete fencing (Lesbarrères & Fahrig 2012) both for amphibians and other small vertebrates. This type of ecopassage is easier to maintain than narrow tunnels and better fits the Hortobágy landscape than high green bridges, although it requires major modifications in the basement of the road. Nevertheless, this is the most cost-effective solution to reduce spatial isolation (Puky 2003). Second, long-term monitoring data such as those collected here should be integrated into road reconstruction plans or new road projects (Garrah et al. 2015). Chapter 4 provided ‘before construction’ data on amphibians for a future before-after-control-impact study, which is the most effective method to measure the effectiveness of a mitigation measure.



9. Általános összefoglaló és gyakorlati alkalmazhatóság

Jelen disszertáció négy fejezetben részletezi a természetvédelmi beavatkozások kétéltűekre gyakorolt hatását. A fejezetek egységes logikai sorrend és felépítés szerint tárgyalják egymás után a kétéltűeket érintő természetvédelmi problémákat mint (i) a fajok pontos elterjedésére és populációik állapotára vonatkozó adatok hiányát, (ii) az élőhelyeik eltűnését, (iii) leromlását és feldarabolódását, illetve az azokat ellensúlyozó tevékenységeket mint (i) a terepi adatgyűjtést (faunisztika, morfológiai elváltozások előfordulása és gyakorisága), (ii) a gyeprekonstrukció kétéltűekre gyakorolt hosszú-távú hatásának vizsgálatát, (iii) a mocsárkezelések békafajokra gyakorolt hatásának vizsgálatát, és a kétéltűgázolások vizsgálatát a Hortobágyon. Az alábbiakban a fenti logikai sorrend szerint részletezem az eredményeket, röviden kitérve a természetvédelmi gyakorlati javaslatokra.

Az 1. Vizsgálat részletes, UTM rendszerben is közölt faunisztikai adatokat szolgáltat a megfigyelt kétéltűfajokról, kitérve a kétéltűek morfológiai rendellenességeinek előfordulására, gyakoriságára, megfigyelt típusaira, és a lehetséges kiváltó okokra (Az 1. Vizsgálat a szerző következő cikkein alapul; Henle et al. 2012; Mester et al. 2015a; Mester et al. 2017).

Az 1. Vizsgálat összesen 11 kétéltűfaj 14 362 egyedét mutatta ki 2010 és 2016 között. A vizsgálat két 10x10 kilométeres UTM négyzet esetében öt (DT86) illetve hét (DT96) faj új előfordulási adataival bővítette eddigi ismereteinket. Nagy állományokkal volt jelen két, az Élőhelyvédelmi Irányelv II. Függelékén szereplő faj, a dunai tarajosgőte (*T. dobrogicus*) és a vöröshasú unka (*B. bombina*).

Az 1. Vizsgálat összesen 11 kétéltűfaj 5596 egyedének vizsgálatán alapul. A morfológiai elváltozásokon belül fejlődési rendellenességek nem voltak jelen, az abnormalitások aránya pedig 0,3% volt, amely nem tér el az irodalmi adatok alapján meghatározott 0-tól 5%-ig terjedő háttér gyakoriságtól, amely általánosan jellemző a természetes



körülmények között előforduló kétéltűpopulációkban. Az abnormalitások közül a hátsó lábak és a farok részleges vagy teljes hiánya fordult elő összesen négy kétéltűfaj (dunai tarajosgöte, vöröshasú unka, barna ásóbéka (*P. fuscus*), zöld levelibéka (*H. arborea*)) és egy fajcsoport (*Pelophylax* kl. *esculentus* fajcsoport) összesen 16 egyede esetében. Jelen vizsgálat elsőként közli farokduplikáció (abnormális, additív farokregeneráció) előfordulását a dunai tarajosgöténél (Henle et al. 2012). Az összes megfigyelt morfológiai elváltozás a ragadozók általi sikertelen predációra vezethető vissza, habár a kétéltűek parazitáinak hatása sem zárható ki. Korábbi adatok híján a vizsgálat nem tisztázta, hogy a fejlődési rendellenességek hiánya és a morfológiai elváltozások alacsony gyakorisága a 40 éve tartó területi védelem és a korlátozott vegyszerhasználat vagy esetleg a közelmúltban végzett természetvédelmi beavatkozások (gyep- és mocsárrekonstrukció) hatására vezethető vissza. A vizsgálat eredményei azonban világosan mutatják, hogy egy védett, a biodiverzitás megőrzése céljából restaurált és kezelt vizes élőhely-komplexum nagy és változatos kétéltű-közösség számára biztosíthat megfelelő életfeltételeket.

Az 1. Vizsgálat eredményei felhívják a figyelmet arra, hogy a természetvédelem számára rendkívül fontos a kétéltűfajok, beleértve a kevésbé ismert, jelenleg még nagy állományokkal rendelkező fajok előfordulásának pontos feltárása és monitorozása, illetve az így szerzett adatok rendszerezése, integrálása és elérhetővé tétele (nyílt hozzáférhetőség) mind hazai, mind pedig nemzetközi szinten. Magyarországon jelenleg számos faunisztikai adatgyűjtő projekt fut párhuzamosan. A munkát a nemzeti parkoktól a civil szervezeteken át számos szinten végzik, de az így szerzett adatok hozzáférhetősége és minősége sokszor eltérő. Ezen adatok összegzése és új módszerekkel (pl. gépi tanulás) történő együttes elemzése a jelenlegi elterjedési adatok pontosítása mellett a jövőbeni trendeket is prediktálhatja és ismeretekkel szolgálhat a kétéltűekhez kapcsolható ökoszisztéma-szolgáltatásokról is, melyek a természetvédelem fontos eszközei lehetnek a döntéshozók



meggyőzésében. A begyűjtött nyers adatok önmagukban nem képesek megvédeni természeti örökségünket (Ellison 2016).

A 2. Vizsgálat eredményei részletesen feltárták a gyeprekonstrukciók kételtűekre gyakorolt hatásait.

Az öt év adatain alapuló ismételt mintavételes elemzés kimutatta, hogy a gyeprekonstrukciós beavatkozás mindhárom vizsgált aspektusa (gyepesítés kora, utolsó termény típusa, felhasznált magkeverék) hatással volt a kételtűekre. (i) Mind a vöröshasú unkák egyedszáma, mind a kételtűek összes egyedszáma és fajsza száma szignifikánsan nagyobb volt a fiatalabb (3-6 éves), mint az idősebb (7-10 éves) gyepesítéseken. (ii) Az összes egyedszám, a pettyes göte, dunai tarajosgöte és vöröshasú unka egyedszám és a fajszám is szignifikánsan magasabb volt az egykori napraforgó táblákon rekonstruált gyepeken, mint az egykori lucernaföldeken rekonstruált gyepeken. (iii) A löszös magkeverékkel vetett gyepeken szignifikánsan több kételtű fordult elő, mint a szikes magkeverékkel vetett területeken.

A tér-idő-helyettesítés (kronoszekvenciák) további különbségeket tárt fel a rekonstrukció rövid- és hosszú-távú hatásai között. Rövid időskálán (2010-ben gyűjtött adatok alapján) szignifikánsan több vöröshasú unka fordult elő az ötéves gyepesítéseken, mint a fiatalabb (2-4 éves) gyepesítéseken, illetve szignifikánsan több unkát fogtunk az egykori napraforgótáblákon és gabonátáblákon végzett gyepesítésekben, mint az egykori lucernátáblák helyén található gyepeken. Hosszabb időskálán (2015-ben gyűjtött adatok alapján) szignifikánsan több barna ásóbéka volt a löszös magkeverékkel vetett gyepesítéseken, mint szikes magkeverékkel vetett gyepesítéseken. Az eredmények alapján a kronoszekvenciák inkább kevesebb vizsgálati évből, de több helyszínről rendelkezésre álló adatsor esetén szolgálnak információval, míg az ismételt mintavételes elemzés több évből, de kevesebb helyszínről származó adatok alapján jóval részletesebben képes megmutatni a gyeprekonstrukció hosszútávú hatásait.



A vizsgálat fontos eredménye volt, hogy a 2010-ben gyűjtött adatok alapján a rekonstruált gyepek nem különböztek a természetes gyepektől sem az egyes fajok vagy fajcsoportok egyedszáma, sem az összes egyedszám, sem pedig a fajszám tekintetében.

Az eredmények alapján a gyeprekonstrukció a gyepek kiterjedésének növelése által hatékony eszköznek bizonyult a kétéltűek védelmében. A mocsarakat és a nagy kiterjedésű vizes élőhelyeket övező és összekötő gyepek a kétéltűek számára zöld folyosókként vonulási útvonalat biztosítanak az egész szezonban, valamint táplálkozóterületként is funkcionálhatnak, a nyári aszályos időszakok, illetve a téli hibernáció idején pedig megfelelő búvóhelyként szolgálhatnak. Ezért a kétéltűek védelmét célzó természetvédelmi beavatkozások fontos részét képezheti a vizes élőhelyeket övező gyepterületek kiterjedésének növelése.

A 2. Vizsgálat alapján a gyeprekonstrukciókat vagy a vizes élőhelyek között, vagy azok természetes vízgyűjtő területén fekvő szántókon célszerű kivitelezni, ezzel létrehozva a megfelelő térbeli kapcsolatot a kétéltűek vizes és szárazföldi élőhelyei között, illetve fenntartva és növelve a szaporodó- és táplálkozóhelyként funkcionáló vizek vízutánpótlását és vízborítottságát.

A 3. Vizsgálat (amely a szerző következő cikkén alapul; Mester et al. 2015b) eredményei kiemelték, hogy a nyár végi nádégetés a következő év tavaszára hatékonyan visszaszorította az előregedett nádat és csökkentette a nádborítást, illetve növelte a mocsári vegetáció változatosságát. Mivel a nád a két évvel korábban égetett területeket gyorsan újrakolonizálta, így az égetéses kezelés a nádfoltok gyors megújulását eredményezte, azaz önmagában nem volt alkalmas a nád hatékony visszaszorítására. Ezzel szemben a szarvasmarhával történő legeltetés hatása hosszabb távon érvényesült a nádborítottság alacsony szinten tartása mellett nyíltvizes foltok kialakítása által.

A kétéltűek fajszáma és összes egyedszáma az átlagos nádborítással és az öreg nád sűrűségével csökkent, míg a nádborítás változatosságával



nőtt. Ehhez hasonlóan a fajszám és az összes egyedszám szignifikánsan magasabb volt a frissen égetett területeken a következő tavasszal. Egy évvel később azonban a fajgazdagság és összes egyedszám a csak legeltetett területeken volt a legnagyobb és jelentősen csökkent a frissen égetett és a kontrol területeken.

A legeltetés és az égetés együttes és jól megtervezett alkalmazásával változatos élőhelyfoltok jöhetnek létre, melyek már a kezeléseket követő tavasszal is megfelelő szaporodóhelyet biztosítanak a kétéltűek számára, míg más élőhelyfoltok jelentősége hosszabb távon, az elkövetkező évek során nyilvánul meg, amikor a szukcesszió hatására a korábban alkalmas élőhelyfoltok megváltoznak. Az eredmények alátámasztják a térben és időben változó élőhelykezelések fontosságát az élőhelyi változatosság és komplexitás kialakításában és fenntartásában a dinamikusan változó tájakban.

A 3. Vizsgálat eredményei alapján három fontos kezelési javaslat fogalmazható meg. (i) Célszerű a legeltetést és az égetést együtt alkalmazni a kívánt célállapot kialakítása mellett (a nádasok egykori élőhelyi és faj szintű diverzitásának visszaállítása) annak fenntartása érdekében is. Ugyanakkor az alacsony legelési nyomással (az itt alkalmazott hektáronkénti 0,6 számosállat-egységet jelentősen nem túllépve) egyrészt alacsony szintre szoríthatjuk a legeltetés kétéltűekre gyakorolt kedvezőtlen hatásait, másrészt növelhetjük az élőhelyek szintjén érvényesülő pozitív hatásokat. (ii) A foltokban végzett, ún. “patch mosaic burning” (PMB) nevű irányított égetési eljárás az érintetlen nádfoltok meghagyásával a kétéltűek számára alkalmas búvóhelyeket biztosíthat az égetés alatt (Parr & Andersen 2006). A mocsarak hosszú távú kezelése során az égetések gyakoriságának ütemezése nagyon fontos, különösen ha időszakos árasztással és legeltetéssel együtt alkalmazzuk (Hackney & de la Cruz 1981; Coops & Hosper 2002; Parr & Andersen 2006; Matthews et al. 2010). A PMB eljárást rotációs módon alkalmazva biztosítható az optimális élőhelyek folytonossága. Például, ha egy adott terület teljes égetését három évente végezzük (igazodva a tüzek



természetes tájtörténeti gyakoriságához), akkor évről évre annak egyharmadát irányított PMB eljárással célszerű kezelni. (iii) Az égetéssel kezeléseket nyár végén, a nád virágzásának csúcsidőszakában célszerű végezni annak érdekében, hogy a nád hatékony viszaszorítása mellett a kétéltűek mortalitása alacsony maradjon. Az eredmények arra is rámutattak, hogy amennyiben a kezelés célja az előregedett, zárt és nagy kiterjedésű nádasok eltávolítása, akkor célszerű az égetést követő télen vagy legkésőbb kora tavasszal az öreg nád rizómáit elárasztani. Ellenkező esetben a nád rendkívül gyorsan visszánő az égetett területeken (Valkama et al. 2008). Az irányított égetéssel kezelések hatékony és biztonságos kivitelezéséhez szükséges ismereteket elméleti és gyakorlati (terepen is) kurzusok megszervezésével kell biztosítani mind az érintett tűzoltóságok, mind pedig a területet kezelő szervezetek, elsősorban a nemzeti parkok érintett munkatársai részére.

Általánosságban véve az égetés és legeltetés együttes, mozaikos elrendezésű alkalmazása hatékony eszköz a nagy kiterjedésű és éveken keresztül felhagyott nádasok fellazítására, amely jól imitálja az adott tájra valamikor jellemző természetes zavarásokat, biztosítva ezzel az egykoron jellemző változatos mocsári élőhely-szerkezetet és vegetációt.

A 4. Vizsgálat eredményei alapján a kétéltűek közúti gázolás általi pusztulásának mértéke a 33. sz. főút hortobágyi szakaszán térben és időben is változik. A legfontosabb térbeli különbség az volt, hogy a többi útszakaszhoz képest az elűtött kétéltűek száma jóval magasabb volt az egyek-pusztakócsi mocsarakkal érintkező úttranszekteken. Az egyek-pusztakócsi mocsarakkal érintkező, összesen 14 km hosszú útszakasz potenciális kétéltű-elütési forrást tekintve a magas forgalom, a megfelelő kétéltűvédelmi infrastruktúra hiánya és az úthoz közeli mocsarakban, csatornáknál, természetes és restaurált gyepeken található nagy kétéltű-állományok miatt. A vizsgálat egyik meglepő eredménye volt, hogy sem a műanyag terelőkerítés, sem pedig a néhol kétéltűalagútként is funkcionáló vízátereszek nem voltak hatással a kétéltűek mortalitására, amely elsősorban kivitelezési hibákra és a



karbantartás hiányára vezethető vissza. A 4. fejezet eredményei szerint a közúti mortalitás az évek között, szezononként és napszakonként is változik. Az évek közötti fluktuációk kapcsolatban lehetnek a csapadékmennyiség változatosságával azáltal, hogy nedvesebb években tavasszal több kétéltűt gázolnak el. A kétéltűek tavaszi szaporodóhelyre történő vándorlását elsősorban az erős kora tavaszi esőzések váltják ki. Ezzel megmagyarázható a 2014 tavaszán tapasztalt kiugró érték, hiszen a felmérések alatt összesen 14,1 mm csapadékmennyiség hullott le. Szignifikánsan több kétéltűt ütöttek el késő tavasszal, mint kora tavasszal. A késő tavaszi felmérés során melegebb és csapadékosabb időjárás volt jellemző, amely ideális körülményeket biztosít, különösen a későn vonuló fajoknak.

A rosszul kivitelezett és karbantartott kétéltűvédelmi létesítmények és a nagy nappali és esti forgalomintenzitás miatt a kétéltűek becsült elütési valószínűsége a teljes vizsgált útszakaszon magas volt. A kétéltűek elütési valószínűsége elérheti a 100%-ot is azokban az esetekben, amikor (i) az egyedek testük felmelegítése (termoreguláció) céljából mennek fel az úttestre, (ii) az úttestet a merőlegesnél kisebb szögben (nagyobb utat megtéve) szelik át, és (iii) két vizes élőhely vagy kerítésszakasz között mozognak az úttesten oda-vissza. Ezek közül sajnos mind a három feltétel fennáll az egyek-pusztakócsi mocsarak mentén is. Az elmúlt 16 évben lassú csökkenést mutatott a forgalom intenzitása, amely az M35-ös autópálya átadását (2006 december) követően méginkább lecsökkent. Ennek ellenére továbbra is nagy forgalmúnak tekinthető a teljes hortobágyi útszakasz, így a kétéltűek gázolási valószínűsége továbbra is magas maradt, főként a nappali és az esti órákban.

A 4. Vizsgálat eredményei alapján a 33. sz. főút egész hortobágyi szakaszán új beavatkozásokra van szükség a kétéltűek közúti mortalitásának csökkentése érdekében. Mivel a 33. sz. főút nagy kiterjedésű vizes élőhelyeket és számos, a kétéltűek számára szintén jelentős szárazföldi élőhelyet (erdőfoltok, gyepek) szel ketté, ezért rendkívül fontos a térbeli elszigetelődés csökkentése. A legjobb megoldás



”zöld átjárók” kialakítása lehet, melyeket az egyek-pusztakócsi útszakasz esetében a lokális kétéltű-elütési forró pontoknál célszerű létrehozni. Azaz e folyosókat az úttest alatt átvezetett csatornák, vízfolyások vagy mocsarak átereszének kiszélesítésével célszerű kialakítani úgy, hogy a folyosó szélessége a keresztező vízfolyásénál mindkét irányban legalább ötször szélesebb legyen (Lesbarréres & Fahrig 2012). A kétéltűek úttestre történő feljutását pedig polimerbeton terelőkkal célszerű megakadályozni (Lesbarréres & Fahrig 2012). Az ilyen kivitelű rendszerek további előnyei, hogy (i) a kétéltűek mellett más gerinces állatok (kisemlősök, madárfiókák, stb.) közúti gázolását is képesek csökkenteni, (ii) a kisebb átmérőjű folyókákhoz és alagutakhoz képest (iii) könnyebb és sokkal olcsóbb karban tartani őket, és (iv) a mocsarak közötti megfelelő hidrológiai kapcsolatok fenntartására is alkalmasabbak. Másrészről azonban az átereszek kiszélesítése bizonyos szakaszokon az úttest töltésének újraépítését követeli meg. Ennek ellenére ez a megoldás biztosítja a leghatékonyabban a vonuló kétéltűek és más állatok biztonságos áthaladását és összességében ez tekinthető a legköltséghatékonyabb és időtállóbb megoldásnak is (Puky 2003).

A megfelelő kétéltűvédelmi rendszerek kiépítéséhez szükség van előzetes felmérésekre (Garrah et al. 2015), melyek eredményeit figyelembe kell venni egy új terelőrendszer tervezésekor, vagy egy új útszakasz megépítésekor (Garrah et al. 2015). Jelen vizsgálat hasznos adatokkal szolgálhat egy esetleges jövőbeni új terelőrendszer tervezéséhez és kivitelezéséhez is. Ezért jelen vizsgálat folytatása nagy fontossággal bír, hiszen így egy új, jövőbeni terelőrendszer létrehozása esetében lehetőség nyílna egy “előtte-utána-kontrol” hatásvizsgálat kivitelezésére, amely a leghatékonyabb módszer az új rendszerek hatásosságának vizsgálatára.



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12. Ethics Statement

Our grassland restoration monitoring studies were part of the After-LIFE follow-up programme (2009-2014) of LIFE-Nature project (LIFE04NAT/HU/000119, <http://life2004.hnp.hu>) and were commissioned and co-developed with Hortobágy National Park Directorate, the main beneficiary of the project. The field data collection protocol, including pitfall trapping, was permitted by the Tiszántúl Environmental Protection, Nature Conservation and Water Management Inspectorate (permit no. 7901/3/2009). Although pitfall trapping inadvertently caused high mortality of amphibian species in the extremely wet year of 2010, when amphibians were more abundant than ever before known by experts, Hortobágy National Park Directorate decided to continue the monitoring studies. Mortality was much less in subsequent years with more normal weather. I regret that many amphibians were inadvertently killed by the pitfall traps, however, I decided to use the data available to gain knowledge for the greater goal of informing the conservation community on the effects of grassland restoration for amphibians.



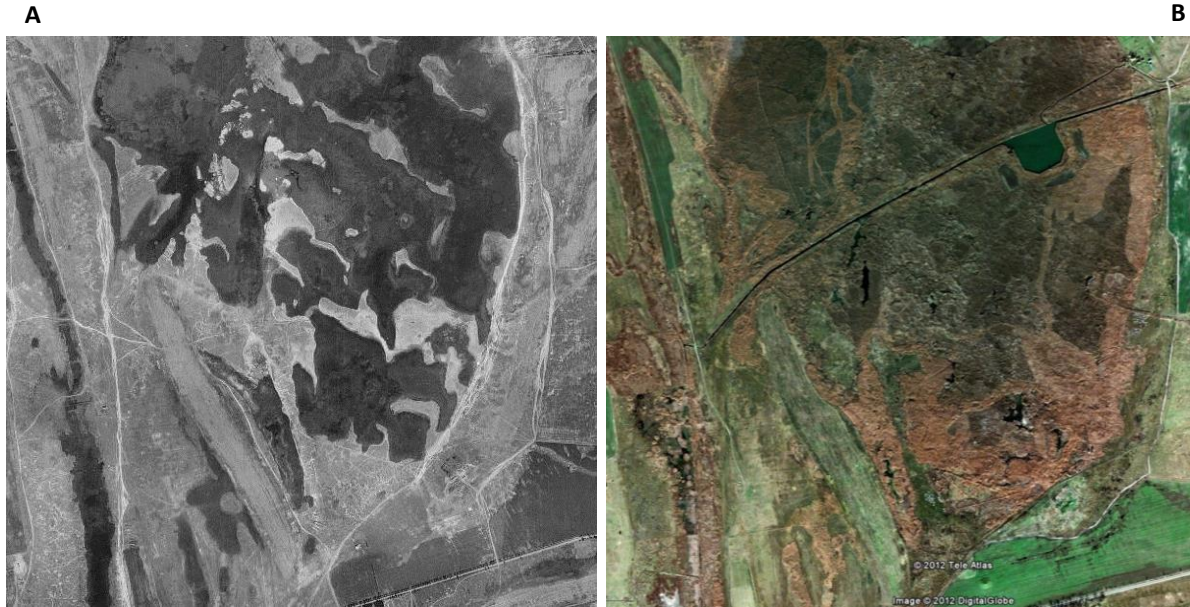
13. Author's contribution – Szerzői hozzájárulás

The studies in this PhD thesis are based on the joint work of numerous participants. Thus, I shortly summarize my personal contribution to the results. In Chapter 1 I performed field and laboratory work, collected field and literature data, sorted and analysed data sets and wrote the manuscripts as first author. In Chapter 2 I performed field-work, collected and sorted the data, performed statistical analyses and wrote a manuscript as first author. In Chapter 3 I designed the study, performed field-work, sorted the data, performed statistical analyses and wrote a manuscript as first author. In Chapter 4 I designed the study, collected datasets, made GIS layers, performed road surveys, sorted the data, performed statistical analyses and wrote a manuscript as first author.

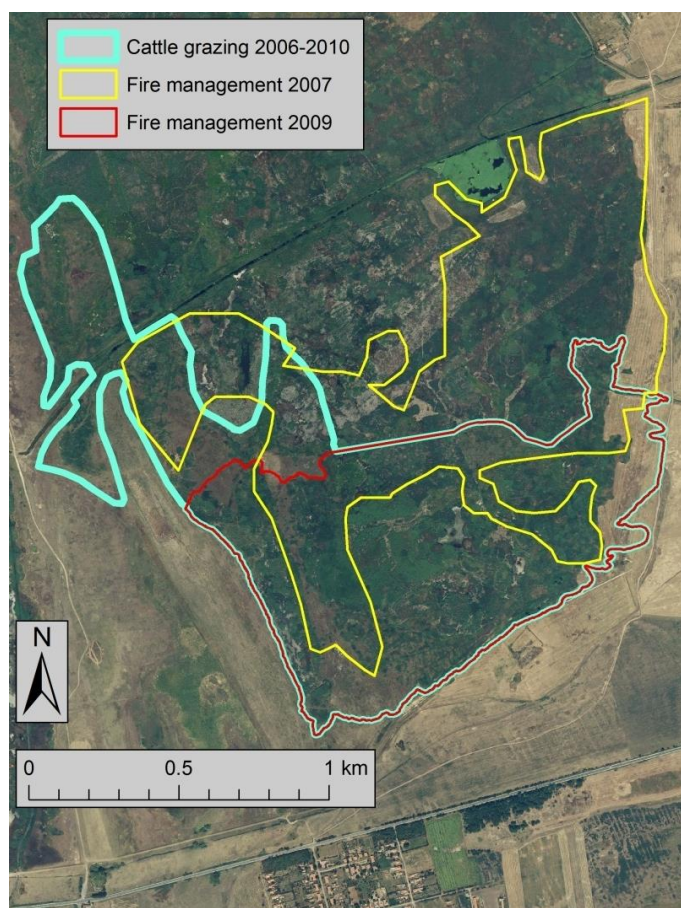
A disszertációmban bemutatott vizsgálatok mindegyike számos ember közös munkáján alapul, ezért a következőkben röviden összefoglalom az eredményekhez való személyes hozzájárulásomat. Az 1. Vizsgálat során a terepi és labormunkákban, az irodalmi feldolgozásban, az adatrendezésben és elemzésben és (első szerzőként) a kéziratok megírásában vettem rész. A 2. Vizsgálatban a terepi gyűjtéseken, a vonatkozó adatok összegzésében, majd statisztikai elemzésében és (első szerzőként) a kézirat megírásában vettem rész. A 3. Vizsgálatban a kísérlet megtervezésében, a terepi munkákban, az adatok rendezésében és elemzésében, valamint (első szerzőként) a kézirat megírásában vettem rész. A 4. Vizsgálatban a kutatás megtervezésében, a szükséges adatok beszerzésében, a GIS fedvények létrehozásában, az útfelmérések kivitelezésében, az adatok rendezésében és kiértékelésében és (első szerzőként) a kézirat megírásában vettem rész.



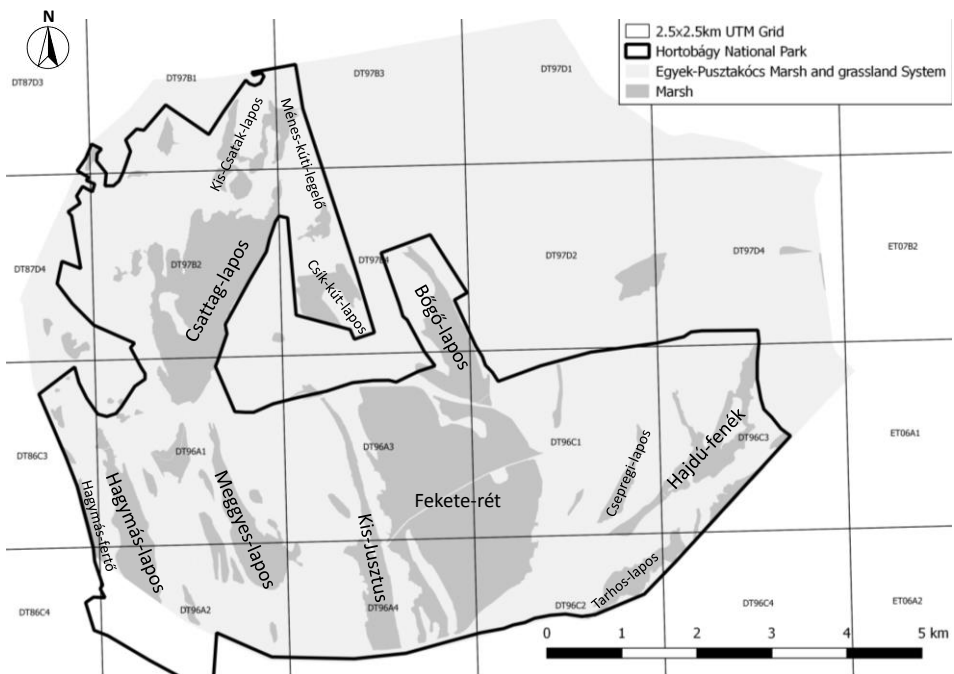
14. Appendices



Appendix 1. Satellite images of Fekete-rét marsh (**A**) in August 8, 1965 and (**B**) in November 2004, i.e., before the start of marsh management in 2004. In the former image bare shorelines, extensive open water surfaces and mosaic vegetation structure maintained by extensive grazing can be observed. Source of images: (A) Institute of Geodesy, Cartography and Remote Sensing, Budapest, Hungary; www.fentrol.hu (accessed 9 December, 2014), (B) Digital Globe & Tele Atlas; Google Earth (accessed 25 March, 2012).



Appendix 2. Aerial image of the study site in 2007, with location of management actions. Source of image: Institute of Geodesy, Cartography and Remote Sensing, Budapest, Hungary.

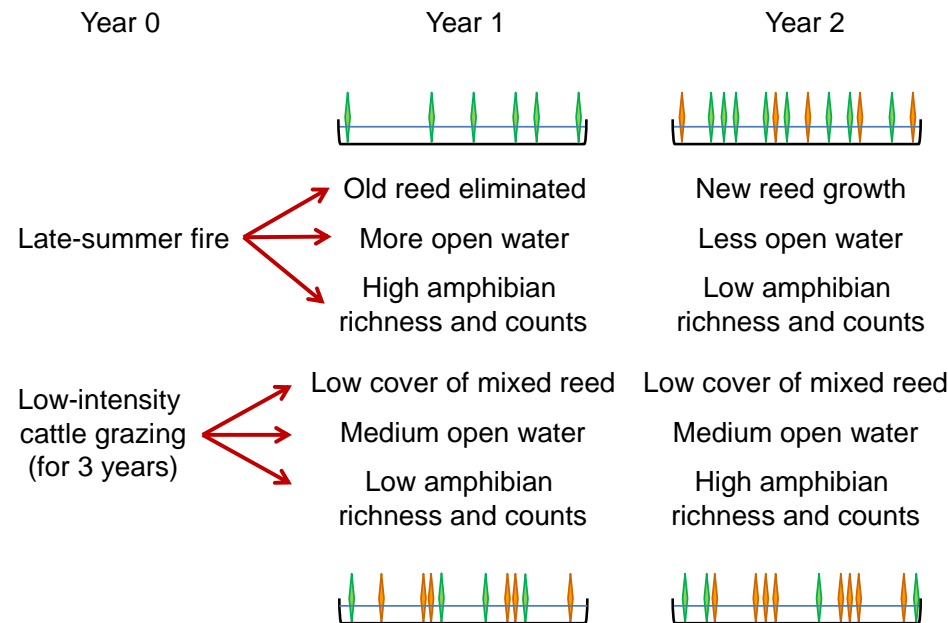


Appendix 3. Map of the 2.5x2.5km UTM grids covering the EPMS with the borderline of the HNP and the name of the large marshes.

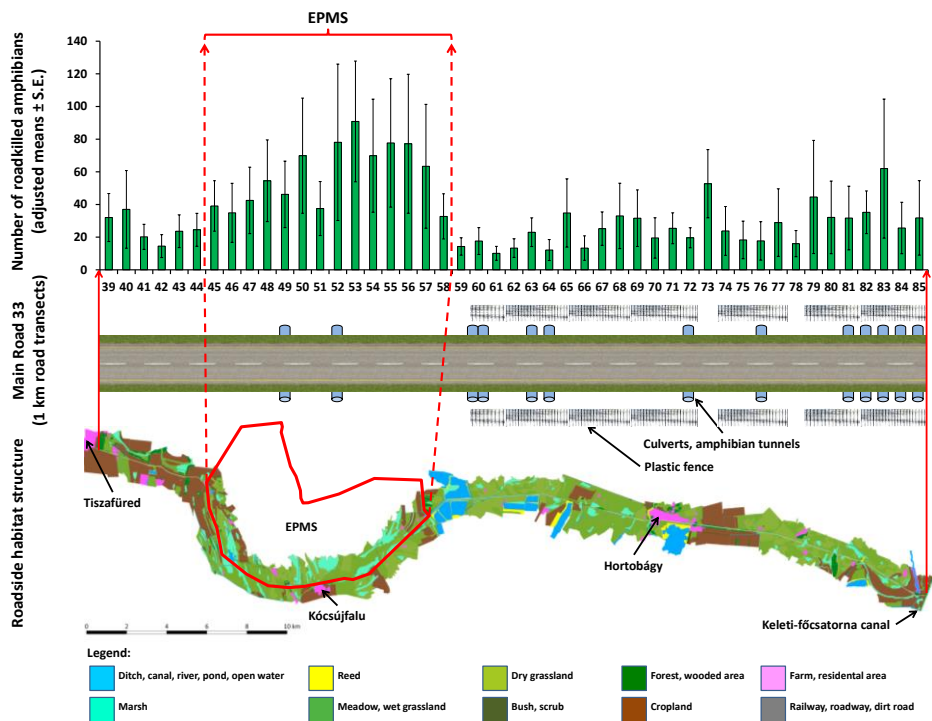


Appendix 4. Pearson correlation coefficients between the proportion of eight habitat types around pitfall traps and factor scores obtained by principal component analyses (PCA). Coefficients used ($r \geq 0.6$) are highlighted in bold.

Proportion of	Restored			Restored and natural		
	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
marsh	-0.641	-	-0.640	-	-0.871	-
meadow	-0.698	-	-	0.303	-0.415	-0.635
grassland		-	0.844	0.891	-	-
wooded area	0.805	-	-	-	0.719	0.347
arable land	0.827	-0.410	-	-0.926	0.312	-
residential area	-	0.691	-	-	-	0.880
artificial pond	-	-0.474	-	0.452	0.389	-
canal	0.601	0.360	-	-0.415	-	0.378
Proxy for	elevation	farms	dryness	naturalness	elevation	farms



Appendix 5. Schematic summary of the effects of burning and grazing on marsh vegetation and anuran amphibians (green - new reed, orange- old reed).



Appendix 6. Number of roadkilled amphibians for all surveyed road transects using five years of monitoring data along the Hortobágy section of the Main Road 33 with the position of plastic fence sections, drainage and amphibian tunnels, the EPMS, and the roadside habitat structure with legends.