THESES OF PH.D. DISSERTATION

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HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

DOCTORAL SCHOOL OF ANIMAL BIOTECHNOLOGY AND ANIMAL SCIENCE

The effect of habitat development and predator management on the dynamics of the hare (*Lepus europaeus*) population

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1. INTRODUCTION AND OBJECTIVES

The brown hare (*Lepus europaeus*) is an animal of the prolific *Leporiade* family, and due to its natural distribution and introductions, it is one of the most ubiquitous mammal species on the planet, allowing it to live in a broad range of habitat types. It was originally a forested gendarme resident, but with the invasion of agricultural regions, it became a species that followed agricultural civilization. Until the 1960s, its estimated stock in Hungary surpassed 1,200,000 specimens, with over 400,000 specimens distributed (Csányi et al., 2021). It found ample, diverse, and high-quality food and hiding places in this anthropogenic, but not intensely cultivated, environment; but, since the heavy cultivation of agricultural regions, its population density has been steadily declining throughout Europe (Edwards et al., 2000). Small game species, for example, can regulate and maintain the vegetation pattern of habitats with their nutrition, they serve as prey for many predatory species, and are thus essential elements of the food web and ecosystems, according to research conducted in recent decades (Viviano et al., 2021).

Hare population dynamics are primarily influenced by ecological variables and usage (Edwards et al., 2000; Smith et al., 2005). External and internal influences, as well as their combined impacts, affect population density (Olesen and Asferg, 2006; Smith et al., 2005). As an internal factor, hare demography is determined by the proportion of reproductive females, which should be at least 70% of reproductively capable females in a healthy population (Jennings et al., 2006; Smith et al., 2005), but in many cases, only 60% of reproductively capable female hares participated in reproduction (Bensinger et al., 2000; Jennings et al., 2006). The yearly reproduction size, which is most typically distinguished by placental scars present in the uterus of female hares (Bray et al., 2003). While the 1970s showed an average of roughly 10 placental scars per female every year (Bensinger et al., 2000; Frylestam, 1979; Kovács and Heltay, 1993), the 1990s showed a decline to 5-6.4 leverets per female (Hansen, 1992; Kovács, 1994). Furthermore, the survival rate of the young and the adult is critical, for which the age must be determined. The existence of the elbow cartilage bulge (Stroh's sign) of the animals is most typically used to estimate the young-to-adult ratio, i.e. the ratio of young individuals who survived until fall compared to those older than one year (Broekhuizen and Maaskamp, 1981; Stankevičiūtė et al., 2011). Furthermore, the eye lens is used as a foundation, which expands with the age of mammals. Based on dried lens masses, there are many age groups (Bensinger, 2002; Stott and Harris, 2006; Suchentrunk et al., 1991).

The habitat, which is continually degrading and diminishing with the development of greyhound regions (Edwards et al., 2000; Panek, 2018), is the most important external factor controlling the population dynamics of the hare. It has been demonstrated that farming with small plots, boundaries, and a diverse structure and composition benefits the hare (Schai-Braun et al., 2020b, 2020a, 2015). The number of hares, the size and diversity of habitat patches, and the flora present in the environment all influence habitat selection (Pavliska et al., 2018). Hare herds are more stable in agricultural regions with diversified flora and mosaics, and arable areas provide a more diverse area throughout the year than grasslands (Mayer et al., 2018; Pavliska et al., 2018). Borders are habitat patches that can link beneficial habitat patches on larger fields as a green corridor, they lower death rates due to poisoning owing to their chemical-free nature, and they provide continuous shelter and a feeding area due to their permanent and more diversified plant cover (Báldi and Faragó, 2007; Biró et al., 2009). The hare's diet is exceedingly diversified (Schai-Braun et al., 2015); however, owing to quick crop rotations and increased chemical usage in intensive farming, the number of food plants vital for hares declines (Biró et al., 2009; Reichlin et al., 2006). External causes such as the growing frequency of extreme weather events as a result of our fast climate change may only contribute to the hare population loss (Rödel and Dekker, 2012).

In addition to deteriorating habitat, the loss caused by predators can drastically diminish hare populations, contributing to a lack of deliberate predator control (Biró et al., 2013; Reynolds et al., 2010). The influence of predators on hare is best described by evaluating hare movement patterns, activity, and habitat use, monitoring stocking numbers, or conducting predator removal experiments. The red fox (*Vulpes vulpes*) is our most significant mammalian predator in terms of small game, and changes in its population are crucial in measuring its influence on the hare (Viviano et al., 2021). External influences include increasing mortality values as a result of agrotechnical treatments (Deák et al., 2021; Karp and Gehr, 2020; Smith et al., 2005) or overexploitation of hunting (Dávid, 2001; Smith et al., 2005).

Last but not least, illnesses (Tsokana et al., 2020), heavy metals (Beuković et al., 2022), agricultural chemicals (Beukovic et al., 2018), and mycotoxin poisoning (Slamecka et al., 2017) external variables like as age (Karp and Gehr, 2020), sex-related mortality, or a drop in reproductive success (Tsokana et al., 2020) can all contribute to a reduction in stock development.

Because the well-being and herd size of small game species, such as the hare, are strongly influenced by the ecological environment, changes in their population characteristics can be used as an indicator of habitat quality, the results of which can be used not only in game management, but also in nature conservation and agricultural greening programs.

In my research, I compared the size of the hare herd to the efficiency of the agricultural support system, vegetation types, field borders, and the degree of predation, all of which can affect the hare's well-being and thus indirectly or directly influence herd development and help the healthy survival of agricultural ecosystems. Furthermore, I demonstrated hare' usage of agricultural areas using night-time reflector stock estimates and GPS telemetry. I correlated the population dynamics of hares captured during hunting with zearalenone mycotoxin that reduces reproductive success.

Because the population of hares is impacted by various environmental conditions, the aims of my studies and research topics can be described as follows:

1. On a local and national scale, I will examine the efficiency of the New Hungary Rural Development Program's Agricultural-Environmental Management Program (AKG) assistance for the hare population.

- Does the amount of AKG-supported land in Hungary impact the estimated and hare harvested density?
- Does the quantity of green areas, as well as the population density and strength of the red fox, impact hare density aside from the AKG subsidy?

- Is the intensity of hare land use (that is, the number of hares seen and the dropping of the hares found) greater in AKG-affected regions than in control areas, i.e. areas not getting support?
- Do the agricultural field edge characteristics and vegetation quality differ between AKG-supported and non-supported areas?

2. In a Jászág sample area, I will use night reflector population estimation to monitor the development of the hare population over multiple years and relate it to yearly changes in vegetation and the success of local red fox control.

- Does the frequency of vegetation in the given region affect the hares' utilization of land?
- Does the reduction of predators affect the hare population? Can the existing vegetation have an impact on this change?

3. Using GPS telemetry, I will illustrate the hare's seasonal and daily use of the area and boundary in the Jászság sample area.

- Can a preference for natural habitat patches or border regions be shown as compared to the inner portions of agricultural fields?
- Is the intensity of hare land use equal to the frequency of offer vegetation supply?
- Do hares choose higher quality border regions, or do they utilize it according on availability?
- Does the minimum convex polygon estimate approach affect the size of individuals and sexes' seasonal and daily home areas?
- Do seasonal and daily home area sizes fluctuate across individuals and sexes according to the Kernel home area estimate method?

Because the dynamics of hare populations in a particular environment are dictated by reproduction and survival in a given year, I had additional goals:

4. I will provide the hare population dynamic indicators derived from a huge number of hunting samples.

- Do autumn sex ratios, young-adult ratios, hare condition, and reproductive characteristics change between areas?

5. Using native placental scar counting and staining, I can collect more precise data to determine the amount of placental scars in female hares, allowing me to estimate reproductive success.

- Is there a difference in the number of placental scars computed natively vs those calculated using the dyed method?

6. I would like to able to detect a probable reproductive issue in female hares by examining the hares' uterus for abnormalities.

- Can a bacterial infection or cytological alteration be discovered in the uterus of female hares with reproductive issues, proving the cause of the problem?

7. To demonstrate and disclose a probable link between the quantity of Zearalenon, a mycotoxin that acts like a mycoestrogen and is found in the organs, and reproductive success.

- Is it possible to identify the mycotoxin zearalenone in hares?
- Can the amount of toxins be linked to female hare reproductive abilities?

8. Finally, using a mixed model, let me demonstrate how the parameters I gathered impact hare reproduction and the proportion of young.

- Does the amount of AKG arable land and green areas impact the number of placental scars in female hares, as well as the proportion of young and breeding females?
- Does the ratio of AKG grassland regions to green areas impact the amount of placental scars in female hares, as well as the ratio of young and breeding females?

2. MATERIALS AND METHODS

1. Analysis of the efficiency of the New Hungary Rural Development Program's Agricultural-Environmental Management Support (AKG)

I chose 13 of the AKG program's 21 target projects that primarily create environmental conservation principles that can have a good impact on huntable and protected small-and medium size animals living in agricultural regions, including the hare. In conjunction with our small-scale study, I chose 13, and in the national-scale survey, 482 game management units (VGE) whose primary source of revenue is small game management, predominantly hares.

In a small-scale survey, I selected parcel blocks (total: mean SD: 4.68 km2 1.52 km2) that are arable or grassland and belonged to the cultivation branch within the (managed) areas belonging to the 13 target programmes, in each VGE, using the Quantum GIS software (QGIS Development Team, 2017). In addition, I chose control plot blocks per VGE that did not get assistance (total mean SD: 3.08 km2 1.78 km2). Because various studies have found that the typical range of movement of hares is about 40 ha (Bray et al., 2007; Zaccaroni et al., 2013), I calculated the treatment plots by making the radius of this 40 ha circle (720 m) the shortest distance between the edge and the border of the control regions. A total of 263 treated and 297 control arable land were chosen for the survey, which was conducted in fall 2013 and spring 2014 along three parallel transects (on the board's edge, 50 and 100 metres inwards from it), totaling 820 km. I conducted a survey using dropping counting (Lioy et al., 2015) in conjunction with flexible strip transect estimate (Thompson et al., 1998). In addition, I counted the number of hares I saw in the area. Because the borders serve as hiding and feeding sites for animals, I graded the agricultural board borders (2 pcs/board) on a scale of 0 to 18 based on their breadth and density. Furthermore, because the quality of the vegetation is also important to the hare, I assessed it on a scale of 0-4. Using a negative binomial, zero-inflated mixed model, I examined the effect of the treatment on the number of counted haress and the number of corpses. I used Sperman's rank correlation to see if the number of hares and corpses is connected to the boundary and vegetation quality.

I used hare density data (calculated and used) as a change in indicator indicators in combination with a national geographic survey. In addition to the proportion of regions supported by the AKG, I considered the fraction of appropriate or favoured sites for hares, as well as statistics on red fox population density. I chose the fraction of arable and grassland areas participating in the AKG subsidy from the 482 VGE, and the proportion of arable land, grasslands, and those green areas that hares prefer ("good areas") from the CORINE Land Cover(Gallego and Peedell, 2001; Pelorosso et al., 2008). I obtained the two VGE population indicators (estimated hare density and hare harvest density), as well as fox estimates and hunting data, from the National Wildlife Management Database (Csányi et al., 2021).

I used linear mixed models (LME) to see if the two population indices differed amongst VGEs in terms of the amount of excellent habitats, the type and proportion of AKG regions, and the intensity of fox decline rates.

2. Hare local population development in a sample region of the Jászág as a function of plant cultural change and predator hunting rate intensity

Between 2013 and 2016, I used the Kovács-Heltay (Kovács and Heltay, 1993) nocturnal reflector stock estimate method in a sample region in Jászság. Distance software was used to calculate population density. To examine vegetation culture preference, I evaluated the area coverage and ratio of the various vegetations for each season and assigned the average number of hares spotted in 3 days with the related standard deviation values. To compute the preference values, I calculated the median value of the observed animal densities per vegetation type and used this to evaluate how much the hare "likes" the distinct vegetation kinds in comparison to each other. In the case of the most frequent forms of vegetation, I also evaluated the vegetation's "preference" based on dropping surveys, and then used the Chi^2 test to compare the hare and dropping density values obtained for each type of vegetation. Prior to the extensive fox reduction measures, I requested and used fox data from the National Wildlife Database between 2007 and 2014, and then computed fox reduction rates as VGE. I used an independent two-sample t-test to compare the differences observed in the sample locations and the surrounding 13 VGEs to the reference year. The yearly den density was estimated to define the fox population in the study region (Heltai, 2016). A professional burrowing team conducted the extensive fox hunting, while local professional hunters in the region used a selective live-trapping and killing trap park to exert extreme hunting pressure on the fox population. To calculate the fox depletion index for the sample region, I utilised the quotient of the density of the exploited fox population and the density of the estimated fox population based on kennel estimations.

I used the "gls" function of the "nlme" programme package of the R statistical programme to investigate whether the calculated hare densities are affected by the season, the fox reduction rate, and the popularity index of the area based on the composition of the vegetation (Fox and Weisberg, 2018).

3. GPS telemetry analysis of the hare

I collected data on 13 hares using a GPS-GSM type telemetry collar to evaluate the animals' more specific territory use and whether the territory use varied daily, seasonally, or between the sexes. Based on sunrise and sunset data, I classified the four localization points collected per day by season, and then calculated the size of the individual's seasonal and daily home range using the commonly used Minimum Convex Polygon (MCP) (Nilsen et al., 2008), as well as the more accurate Kernel home area estimate (KDE) (Frate, 2022; Tóth et al., 2014). We investigated the status of the agricultural crops in the region, as well as the natural habitats and margins, in both the late fall/winter and spring/summer seasons.

I aggregated the total winter, spring, night, and day localization points of the hares placed inside the telemetry region and created as many randomly dispersed points as there were localization points in the given time to estimate the preference of the places. The χ^2 test was used to assess the differences between the obtained values. I utilised the R program's "RVAideMemoire" programme package to do a "Bonferroni" adjustment when the hares did not use the region randomly. Using the Ivlev index, I estimated the popularity of various cultures. I also used the $\chi 2$ test to examine the intensity of utilisation of natural habitats and linear borders with randomly distributed points, depending on border vegetation and border quality.

Finally, I calculated the strength of the experience localization points' edge utilisation as follows: I computed the frequency of the points by generating 20-meter bands from the borders of the edges buffered by the transmitter error towards the core of the sign. Then, using the $\chi 2$ test, I compared the generated distribution to the uniform distribution that we would receive if the hares did not favour edges.

4-5. Collecting and analysing hare population dynamic indicators

Using native and placenta scar staining procedures, I collected samples from 29 hunts of 9 VGE (a total of 1007 hares) between 2013 and 2017 to examine the young-to-age ratio, condition, sex ratio, and reproductive parameters of the individuals in different areas and between years.

The ratio of the weight of the left kidney to the weight of the fat layer around the kidney (kidney fat index VZsI) was used to determine the condition of the hares. Individual age was established based on dry lens weight, then divided into young/old groups (Kovács and Heltay, 1993), more specific categories within one year, and ultimately years past (Andersen and Jensen, 1972; Bensinger, 2002; Suchentrunk et al., 1991). I counted placenta scars to establish the reproduction rate per female. I examined the uterus of all females natively and after the multi-step placental scar staining method, as Bray et al. (2003) did.

Using variance analysis, I compared the native placental scar counts to those acquired by the staining process. Furthermore, numerous population dynamics indicators were compared between hunting years, sexes, and reproductive success.

6-7. Investigating the hare's breeding issues

I sent samples from the uterus of the abnormally looking female hares for veterinary examination in order to confirm bacterial infection or cytological changes occurring as a secondary consequence in the uterus, which may cause the reproductive problems.

Since other chemicals and toxins that affect the endocrine system can also cause reproductive problems (Paterson and Lima, 2011), I measured the amount of a mycoestrogen, zearalenone (ZEA) (Gromadzka et al., 2008), which has not yet been studied in hares, in different organs of hares, and I compared the found ZEA amounts with the oestrogen and progesterone hormone contents of the individuals'. I also applied linear regression models to investigate the association between the ZEA level and the amount of placental scars. Finally, I conducted an independent two-sample t test to evaluate ZEA and hormone levels in people with problematic and normal uteri, as well as reproductive and non-reproductive females.

8. Investigating the hare breeding indicators based on the varied features of the VGEs

I used a zero-inflated mixed model to study how the number of placental scars varies by animal age (young or old), the quantity of arable land/grassland and its AKG coverage, and the fraction of "green areas" on the VGE. Using the "gls" function of the R "nlme" programme package, I analysed the analysis units, the proportion of problematic bees and non-reproducing females, and the proportion of young individuals.

3. RESULTS AND THEIR DISCUSSION

1. Evaluation of the efficiency of the New Hungary Rural Development Program's Agricultural-Environmental Management Support (AKG)

At a small geographical scale, AKG had no influence on the amount of dropping in any season or cultivation branch. We discovered less dropping in the spring than in the autumn, and the density of dropping was also lower in arable fields than lawns. The zero-inflated component of the model revealed that as the height of the vegetation and the extent of the studied area increased, so did the detection inaccuracy of the debris. In the autumn, there was no difference in the number of hares between the AKG and control arable lands, but in the spring, the AKG arable lands had much more hares than the control. We spotted more hares in the control areas of lawns in the spring. However, we did not discover hares in the majority of the agricultural fields we investigated. The detection error of hares did not vary with season, although it was lower in bigger agricultural plots, greater vegetation, and less thick field edges (Figure 1).

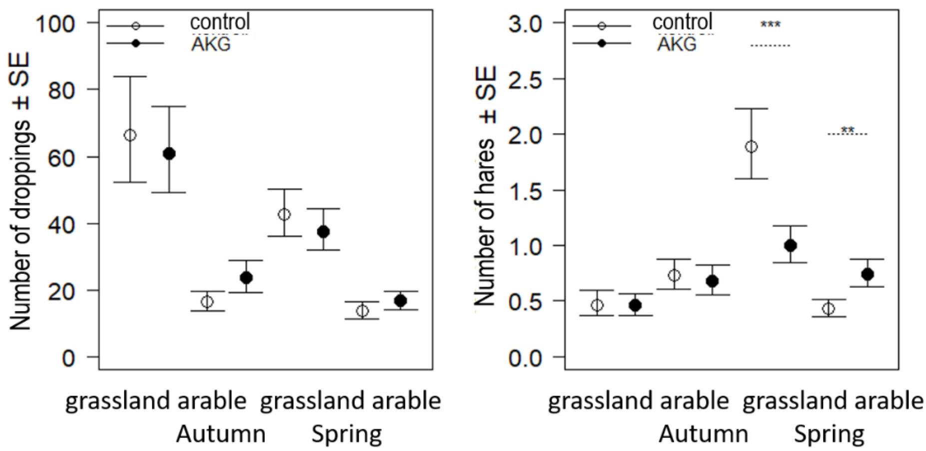

Figure 1: The comparation of hare droppings and observed hare populations. The error bars are mean and standard deviation values calculated from the model and translated back to the original data scale. Asterisks indicate that the groups are substantially different (**P 0.01, ***P 0.001, following FDR correction).

AKG borders were substantially superior than control grasslands in terms of quality (Mann-Whitney W=5316, P=0.009). In the case of arable land, I discovered no discernible difference. The median value of edge quality was poor across the board. When identifying the vegetation, I saw no significant difference between the AKG and control fields, despite the fact that the AKG fields had higher border quality scores on average. AKG grasslands, on the other hand, showed considerably worse vegetation quality than control grasslands (W=8668, P 0.0001). I discovered a negative relationship between vegetation and border quality (N=642, Rho=-0.12, P=0.001): the border quality of the control grasslands was substantially greater than that of the fields (W=13733, P=0.019) (Figure 2).

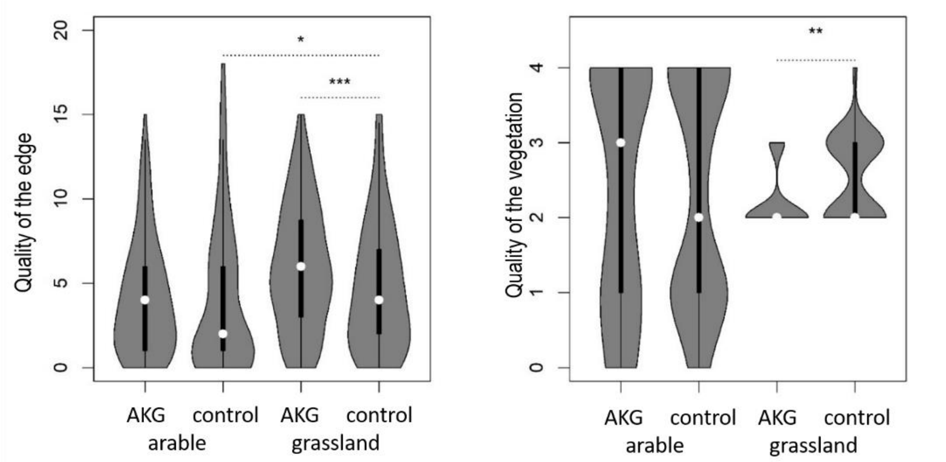

Figure 2: Field edge distribution and vegetation condition in the AKG and control areas. The violin plot depicts the data's Kernel density function; the thick line represents the interquartile range, and the white circle represents the median. After FDR correction, asterisks indicate a significant difference (*P 0.05, **P 0.01, ***P 0.001)

The vegetation on the AKG grasslands was worse, while the border quality was superior to that on the control grasslands. I discovered a higher dropping density in the grasslands than in the fields, which could indicate that animals spend more time resting on the grasslands or that there are more grass species suitable for hares (Schai-Braun et al., 2015), even though hares avoid heavily grazed areas (Schai-Braun et al., 2013). Although the borders of the AKG sections were of greater grade, they were not spectacular.

The estimated hare density index fell year after year in a countrywide study. There were no more animals found in the "better areas" overall. According to the model, if the game management units had a high percentage of AKG arable land, the anticipated hare density would increase. At the same time, if there was a high proportion of AKG fields but the fox reduction rate in the region was low or average, the hare population would be unable to rise. Animal density declined somewhat, although in a trend, as the number of AKG lawns increased. Hare distributions have shrunk through time, particularly in places with a high percentage of suitable habitat. The utilised hare density remained consistent year after year in those VGEs with a larger proportion of AKG grassland. The fox's decrease rate had no influence on the rise in cover density.

Overall, it appears from a national spatial scale study that even in less good areas, the support could not improve the habitat because its effect did not depend on the proportion of good areas, i.e. even in the case of VGEs with a small proportion of good areas, it could not add anything to the hare population density a lot of AKG. Cattle pasture made up a sizable portion of the AKG grasslands. Because the effect of intensively grazed regions on hares is manifestly unfavourable owing to excessively short grass (Fourcade et al., 2018), the high standard deviation values reported in the case of high proportion AKG grasslands may be explained by this.

2. Hare local population development in a sample region of the Jászág as a function of plant cultural change and predator attack intensity

When looking at the autumn densities, a rising tendency may be detected throughout time. When comparing the spring core densities, I too noticed a growing tendency until 2015, when the densitie dropped. Due to unfavourable weather circumstances in the spring of 2016, stock estimation with night reflectors could only be performed later, in April, implying that the spring of 2016 displays an underestimation (Figure 3).

According to dropping densities, hares prefer lucerne and avoid recent planting of grain, sunflower stubble, ploughing and dialled corn. Sedges, lucerne, rapeseed, dialled regions and stubble and grassland are essential habitat patches for the hare based on plant preferences. Animals live in a range of settings, and their occurrence, with a few exceptions, follows the frequency of the particular plant type. Hares avoid maize, sunflowers, and ploughing, despite the fact that these types of flora cover 40% of the land.

According to the model, hare population density is mostly determined by the degree of fox predation. The influence of season and vegetation is insignificant, however the extent of the fox reduction has a positive effect on hare population development. At the same time, the greatest herd size could be seen when the fraction of favoured plants in the region was greatest and the fox reduction rate was greatest in the spring of the given year. Thus, the local hare density is affected by both the severity of fox predation and the attractiveness of the region, although the latter alone is insufficient.

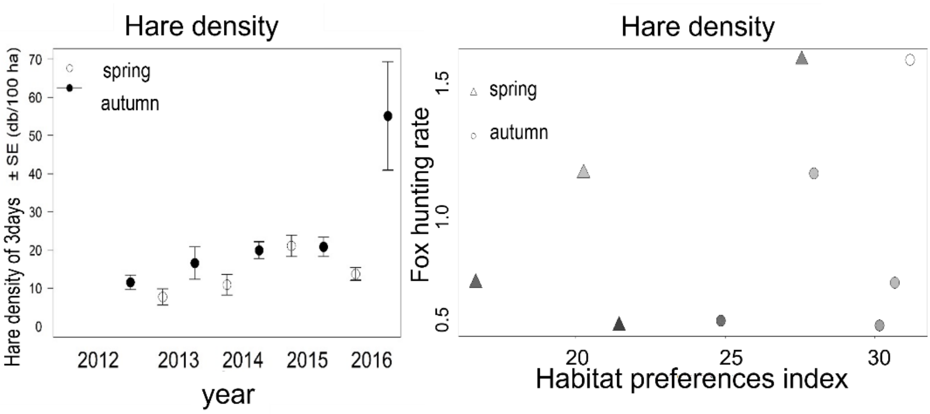

Figure 3: The hare densities of the stock estimation using night reflectors (left picture), the evolution of the hare density as a function of the area preference index and the fox reduction rate. Each circle or triangle symbolises a distinct year. The hue of the symbols shows the degree of hare density: a lighter colour suggests a higher density

3. Hare GPS telemetry investigation

The distribution of hare measured localization locations did not differ substantially from the random distribution, implying that hares use the same region as the frequency of current vegetation supply. According to the Ivlev index, hares obviously preferentially used the homestead, fallow, and sunflower stubble in winter. Only fallow was favoured in the spring, whereas spring grain, wild land, corn and ploughing were less utilised than in the steady state. Alfalfa was classified as recommended based on the KDE home range, whereas it was classified as avoided based on the larger MCP home range.

The utilisation of different types of natural habitat and edges of varying quality did not differ from random use. Simultaneously, the great majority of the localization sites were clustered along the margins, and their frequency declined as they travelled towards the core of the field, indicating a preference for the edge areas over the central portions of agricultural fields.

I found no significant variations in seasonal and daily range of motion using the 95% and 60% MCP and the 95% KDE home range estimate methods. At the same time, animal activity at night was higher on average than during the day. According to the 95% KDE, females used a bigger area in the spring and during the day than bucks. I was able to detect greater migrations (more than 4 km) and a shift in territory in the case of two females.

4-5. Hare population dynamic indicators based on data collected

Overall and by year, I discovered a statistically insignificant but minor sex ratio with female weight, which is similar with the findings of previous research (Farkas, 2021; Slamečka et al., 2014). The proportion of youngs in the studied samples was 43%, which was consistent with the findings of other research (Bensinger et al., 2000; Beuković et al., 2013; Popovic et al., 2015). The majority of young in a particular year who survived the hunting season were born between April and August. The proportion of individuals born in the first quarter of the year was low, and I found none of the individuals born near the end of summer. Only 2.4% of the samples were between 1-1.5 years old, despite the fact that this is the age group most likely to participate in reproduction. The age group over 5 years with the lowest reproductive potential made up about a quarter of our sampl (Figure 4).

The animals' VZsI values ranged from 0 to 13, with a substantial variation across years and sexes. The greatest VZsI levels were seen in adult male hares (Figure 5).

Females with abnormal genitalia had a higher VZsI value (W=37260, P=0.02), independent of age group, although there was no difference between the condition of females engaging in reproduction and not participating in reproduction.

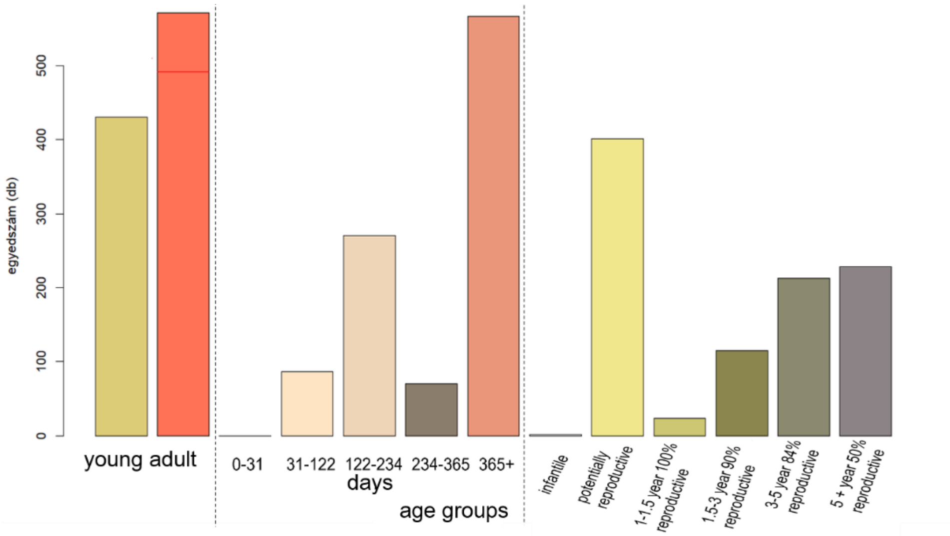

Figure 4: Age group evolution based on several groupings: young and elderly, distributions within a year and between years, and reproduction rates

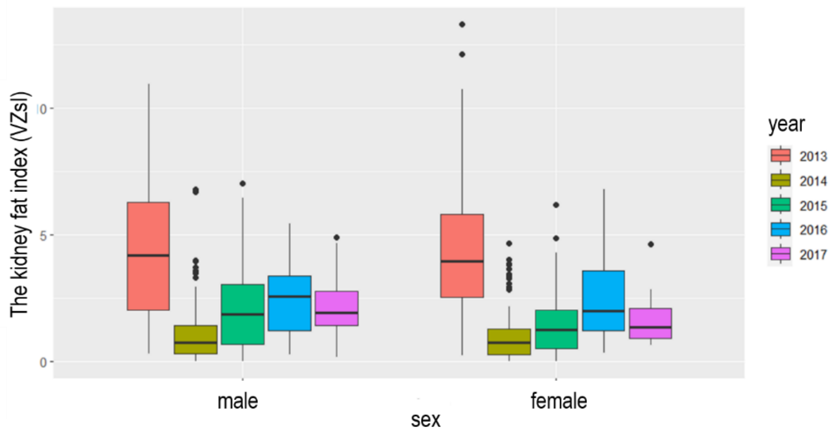

Figure 5: Evolution of VZSI levels in males and females throughout years

I discovered 117 individuals with genital abnormalities out of 492 females. For possibly fertile females, the average number of placental scars was 7.34 (6.12) SD. The number of placental scars encountered in the study locations varied substantially (df=8, F=2.48, P=0.013), and I computed greater placental scar counts using Bray et al. (2003) placental scar staining technique (Fig. 6).

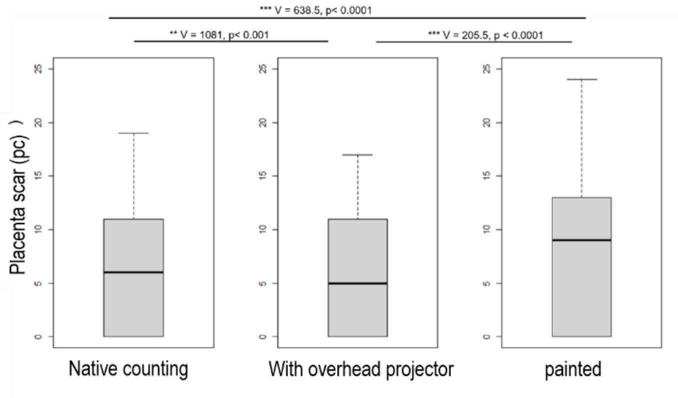

Figure 6: Changes in placental scar counts as a result of different staining processes (* denotes a significant difference; significance levels may be inferred from the picture)

6. Correlations of a suspected reproductive issue in female hares demonstrating the disease

37% of females that survived at least one winter had genital alterations, whereas 57% did not reproduce at all. Similarly to Gál (2006), I discovered females in whose placental scars could not be recognised, despite the fact that the condition of development of the genital organs would have allowed this. The oldest age group had the highest prevalence of abnormal genitalia (43%). Only five of the 45 submitted samples had a *purulent inflammatory* origin with *epithelial hyperplasia*, whereas 10 indicated *epithelial* cell, *mesenchymal* cell, *epithelial*, *histiocyte*, or *fibroblast poliferatio*. There were no cytological abnormalities observed in the 21 instances, and no mixed colonizing/contaminating flora or intestinal bacteria were cultured. In many cases, inflammation or other alterations were not proven (maximum connective tissue sprouting), or just a limited number of inflammatory cells were found, which does not explain the infertility. At the same time, because the samples were sampled from frozen animals rather than freshly hunted animals, a sampling error is possible.

7. Zearalenone levels discovered in organs and their putative relationship to female reproductive disorders

I was able to effectively identify ZEA in hare kidneys and livers. The mycotoxin was not found in the muscular tissues (tongue) tested. I found that the ZEA levels in the young people's bodies were higher (Figure 7).

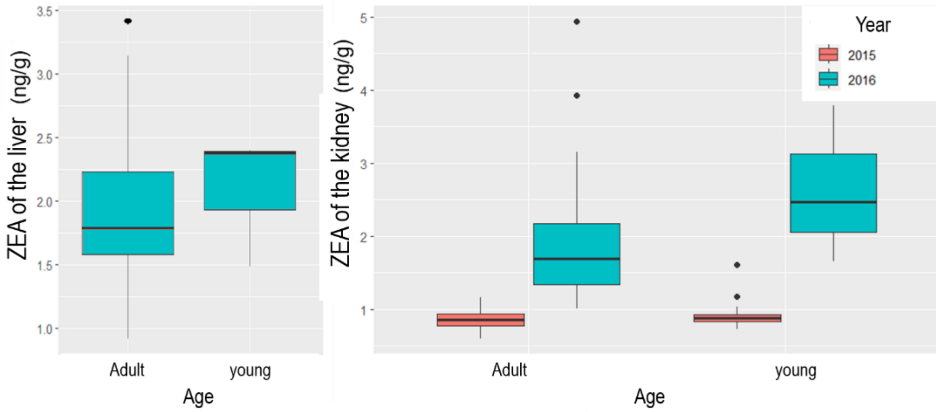

Figure 7: Changes in ZEA levels in the liver (left) and kidney (right) throughout time and age groups

I found a correlation between the ZEA content of the kidneys and the quantity of E2 in the ovaries. The amount of placental scars reduced as kidney ZEA content increased. The ZEA content of kidneys from people with problems genitalia was substantially greater (normal average 1.18, problematic average 1.65, t=-2.51, df=52, P = 0.015), but livers and intestine corners did not vary. Females with normal uteri had substantially greater E2 hormone levels in their ovaries (normal mean 0.37, problematic mean 0.3, t=2.07, df=64, P=0.042). There was no change in P4 hormone levels between problem and normal youngs.

Females who did not reproduce had a higher ZEA kidney value (reproduced 1.26, non-reproduced 1.7, t=2.05, df=43, P=0.046). The amount of E2 hormone in breeding individuals was significantly higher (breeding average 0.38, non-breeding average 0.25, t=-4.26, df=59, P 0.0001), indicating that the current ZEA amount cannot increase the amount of E2 hormone as much as the level of reproductively active females, but it can slightly shift the ratio.

In terms of hormones, females with higher E2, non-reproductive individuals, or individuals with certain genital abnormalities had higher ZEA levels in their kidneys. The number of placental scars per female reduced as the level of ZEA in the kidney rose. Because a major portion of the mycotoxin is expelled with the fecal, no association could be proven; nevertheless, in the case of lagomorph, due to *cecotrophy*, these animals were considerably more vulnerable to the hazards of ZEA.

8. AKG areas in fields/grasslands and the influence of green space on reproduction, reproductive success, and the proportion of young

According to models, the fraction of AKG regions has no effect on reproductive success, or whether the hare reproduces. The proportion of grasslands grows, while the proportion of arable fields decreases reproductive failure. The high quantity of arable land and widespread AKG coverage enhance the number of placental scars, i.e. the number of leverets born, as well as the proportion of newborn hare surviving the first year. In contrast, increasing the quantity of grasslands diminishes it.

4. CONCLUSIONS AND RECOMMENDATIONS

1.) The impact of the AKG support system on hare population development

Overall, it was not able to show that the AKG Programme had a substantial influence on the hare population from 2009 to 2014. The AKG had a good effect on border quality in studies - but it is also possible that places with greater border quality benefitted from the subsidy - but the programme did not increase the quality of vegetation in fields or lawns. Because a telemetry test proved that hare movement activity is greater at night, counting hares during the day is insufficient to determine the performance of such programmes, but when combined with corpse counting, it may sufficiently identify the differences.

Small-scale and telemetry investigations have also showed that the proportion of arable ground in the winter is relatively high, despite the fact that the animals prefer it less. In the winter, vacant arable land might be supplied by spreading green manure plants, which help improve the soil's microbial population. In such instances, species selection is critical since some plants are avoided by animals and vegetation taller than half a metre can also be a physical barrier for animals (Mayer et al., 2018). If this is not practicable, we should aim to broaden the edges, such as by planting 60-

meter-wide stubble leaves or establishing bee grazing strips on field boundaries (Marshall et al., 2006).

In many cases, the efficiency of AKG programmes can only be determined after they have been in operation for an extended period of time. In the context of a national scale investigation, it is possible that the data from the selected reference years still represented the beneficial influence of past AKG support, boosting the reference level. In the future, a large-scale research would be beneficial to investigate the impacts or combined effects of additional subsidies comparable to AKG, which should be able to adequately explain the influence on the hare population. Furthermore, it would be worthwhile to consider whether, under current regulations, increased AKG coverage would have a measurable positive effect, or whether it would be more beneficial to shift to result-oriented support projects that measure the success of support systems using bioindicators (Concepción et al., 2020; Kleijn and Sutherland, 2003).

2.) Predators and the impact of the hare's existing environment on its behaviour

Based on telemetry data, it appears that both habitat enhancement and predator management must be planned and performed on a broader spatial scale, as the targeted hares' range of movement was substantial, contrary to the literature data. Larger mobility areas may also be caused by the poor diversity of habitats that arises as a result of large-field farming. The exact environment and vegetation appear to be not equally significant for the two sexes. Natural habitat spaces are critical for animals; all of the identified hares utilised such sites.

The telemetry study taught us that defining and categorising each vegetation based on satellite recordings or a single recording is insufficient because, for example, groundwater can create a completely new microhabitat in different parts of the agricultural field, which can be critical for the hare. This will be done professionally in the future by filming or more precise mapping, such as drone recording.

Varied estimating methodologies might result in drastically varied dwelling area measurements. Thus, in the future, in addition to the most commonly used minimum convex polygon estimation, it would be desirable to additionally do the Kernel home area estimation, which indicates an area half as big, especially if the individuals make a longer journey.

Some female hare may alter their territory periodically. The size of the field is clearly important from the perspective of the animals, as the majority of the localization points fell around the edges in each season, and moving towards the interior of the field, their frequency decreased, and the interior of the large fields was not used at all. Regardless of their quality, the animals employed the edges based on their frequency of occurrence, however it is conceivable that the defined categories did not reflect the hares' true preference characteristics. Due to the intensive usage of edges, the night reflector estimation must be altered in the future since one of its requirements, which requires that the distribution of all individuals be random, is not met. Because the great majority of estimate routes travel along the outskirts, we will induce an overestimation, which will be a big concern in the event of a species with a falling stock tendency. This may be improved in the future by fixing the formula.

Our experiments demonstrated that the fox reduction index has a demonstrable favourable influence on hare density, although it is insignificant on its own. According to a small-scale study, the maximum hare density could be achieved when the fraction of favoured plants in the region was the highest and the fox decline rate was likewise the highest in the spring of the given year. Thus, both the intensity of fox predation and the preference of the location influence hare numbers. According to my findings, rigorous predatory thinning can boost hare population, but it can only be sustained by sufficient quality habitat. To explore the fox's more subtle influence on the animal, more experiments based on indirect signals (feeding leftovers, hare hair detected in poo) should be conducted in the future.

3.) The evolution of the hare's reproductive features, reproductive capacity, and challenges in relation to habitat

The winter stock sex ratio reveals a modest but female predominance, which is not unique to Hungary (Slameka et al., 2014). Females that do not reproduce or have defective genitalia do not need to devote extra energy to their litters, allowing them to prepare for winter in better shape. At the same time, metabolites of numerous substances and poisons accumulate in fat (Beukovi et al., 2022; Beukovi et al., 2018), which should be researched more in the future, as this may further lower individuals future reproduction rate.

The amount of yearly reproduction and the frequency ratio of problem bees appear to vary greatly from area to area and year to year, so you should not generalise or make major conclusions based on just one year or a few hunts in one location. Since Bensinger (2002) states in his thesis that in the 1970s, the proportion of females with reproductive problems or infertility was only a fraction of what it is now, with a higher proportion of young, it is possible that the introduction of newer agricultural chemicals, their accumulation, or the increasing frequency of extreme weather events drive the hare population downward. As the frequency of abnormal uterus increases to nearly 50% over the age of 5, the proportion of young in the population decrease (Beuković et al., 2013; Ristic et al., 2020), with more than 15% also having abnormal genitals, increasing the likelihood of infertility or a low birth rate. That is why it would be worthwhile to conduct a nationwide monitoring, at least on the basis of sex ratio, young-old ratio calculated from Stroh's sign, and body weight, to see which areas have a bigger problem, and whether these ratios change between years, and if so, in what direction. This would be an excellent starting point, which could be reinforced with vegetation data from the areas or weather conditions to discover what factors restrict juvenile survival or which types of vegetation can effect reproductive success. Later, it would be suggested to collect samples from locations with a larger number of problematic or non-reproductive females, reproductive healthy females, and adversely performing females in order to determine the quantity of ZEA in their kidneys and hormone levels in their ovaries. As a result of local research and national monitoring, large-scale interventions and treatment plans for both European-level species management and agricultural programmes might be developed in the future.

5. NEW SCIENTIFIC RESULTS

1.) In Hungary, I studied the influence of the ÚMVP AKG programme on hares at the national and small geographical scales.

• In our small-scale survey, I was unable to show a beneficial influence on hare density, regardless of support, because the majority of the edges were of low quality.

• On a national geographic scale, I was able to establish a strong connection between fox density, thinning rate extent, and AKG arable land expansion.

2.) I was able to objectively validate the joint influence of vegetation and intensive fox hunting on the establishment of local hare populations.

• I demonstrated that the density of the hare population is primarily determined by the intensity of fox predation, which can be confirmed by the presence of favourable vegetation.

• I demonstrated that orphan crops, lucerne, rapeseed, dialled areas and stubble, and lawns are important habitats for hares using night-time reflector data collected over several years. I showed that animals employ a wide range of environments, and their occurrence is mostly determined by the frequency of plant kinds.

3.) I was the first to use GPS-GSM type telemetry testing with the species in Hungary, and I compared several home range estimation methods from the collected data.

• I demonstrated that in our country, compared to the 30-40 ha home range found in the literature, individuals can use a much larger range of movement, the home range of females can exceed that of males, and I was able to demonstrate a change in territory.

• I showed that, while the generally used 90% MKP estimating approach is less accurate, the 60% MKP or 90% KDE home area estimate is more accurate.

• I've proven that hare utilise the borders and areas of the field near the borders more intensely, independent of quality, thus the processing approach of stock estimation using reflectors at night must be adjusted in the future to avoid overestimation.

4.) We were the first to develop procedures for detecting the concentration of ZEA in hare organs. I was able to demonstrate the presence of the poison in a hare's liver, kidney, and intestine.

• I demonstrated that females with abnormal genitalia had a higher kidney fat index.

• I found that the quantity of the E2 hormone rises with the amount of ZEA in the organs, implying that ZEA may be implicated in the development of reproductive abnormalities.

• Despite the fact that the uterine mucosa wall was plainly swollen, I was unable to discover the presence of a pathogen or inflammation in the issue bees, which would indicate a reproductive problem.

5.) For the first time in our nation, I compared the commonly used placenta scar counting approach to a multi-step staining procedure and found a difference in favour of the latter. As a result, it is thought that the figures recorded in our country thus far are understated.

6.) Using a large number of samples, I discovered that regardless of the year, the proportion of young people born in the first quarter of the year and at the end of summer is low among those who lived through the hunting time. I shown, using a multivariable statistical model, that the extent of arable land and its AKG share can have a favourable influence on reproduction and young survival. Increased arable land may help lower the number of abnormal utherus. Green spaces cannot have a good impact, but their proportion per area was fairly low.

6. LIST OF PUBLICATIONS RELATED TO THE DISSERTATION'S TOPIC

Published and accepted publications:

Ujhegyi, N., Keller, N., Patkó, L., Biró, Z., Tóth, B., & Szemethy, L. (2021). Agri-environment schemes do not support brown hare populations due to inadequate scheme application. *Acta Zoologica Academiae Scientiarum Hungaricae*, *67*(3), 263–288. (IF: 0,91)

Szakács, Sz., Ujhegyi, N., & Biró, Z. (2020). A mezei nyúl populációbiológiai jellemzői a Jászságban. *Vadbiológia*, *20,* 33-42

Patkó L., Ujhegyi N., Szabó L., Péter F., Schally G., Tóth M., Lanszki J., Nagy Z., Szemethy L., Heltai M. (2016) Even a hair casts its shadow: Review and testing of noninvasive hair collecting methods of carnivore species. *North-Western Journal of Zoology*, 12, 130–140. (IF: 0,87)

Patkó L., Ujhegyi N., Heltai M. (2016) More hair than wit: A review on carnivore related hair collecting methods. *Acta Zoologica Bulgarica*, 68, 5–13. (IF: 0,53)

Ujhegyi, N., Biró, Z., Molnár, Z., Keller, N., Patkó, L., Tóth, B., Kovács, I., & Szemethy, L. (2015). Az agrár-környezetgazdálkodási program hatása a mezei nyúlra (Lepus Europaeus) Békés megyében. *Tudomány És Innováció a Lokális És Globális Fejlődésért*, 65–74.

Ujhegyi, N., Biró, Z., Patkó, L., & Keller, N. (2015). Élőhelyfejlesztés és ragadozógazdálkodás hatása a mezei nyúl (Lepus europaeus) populációdinamikájára. *Természetvédelmi Közlemények*, *21*(1), 362–372.

Szemethy, L., Keller, N., Ujhegyi, N., Csányi, S., Kovács, I., Patkó, L., Schally, G., Tóth, B., & Biró, Z. (2015). Az apróvad, mint az agrár-környezetgazdálkodási programok hatásindikátora– módszertani áttekintés. *Tájökológiai Lapok*, *13*(1), 9–17.

Citizen science publications:

Keller, N., Biró, Z., Szemethy, L., Farsang, Z., Ujhegyi, N., & Csányi, S. (2019). A mezei nyúl területhasználata újabb adatok tükrében. In L. Dr. Jámbor (Ed.), *Vadászévköny* (pp. 43–50). Dénes Natur Műhely.

Szemethy, L., Ujhegyi, N., Keller, N., & Biró, Z. (2018, February). A mezeinyúl-gazdálkodás fejlesztésének lehetőségei a kutatások tükrében. *Nimród Vadászújság*, 4–8.

Szemethy, L., Biró, Z., Heltai, M., Patkó, L., Schally, G., Szabó, L., & Ujhegyi, N. (2014). Nyúl viszi a... sast. Vadászok a parlagi sasért. In J. Pechtol (Ed.), *Vadászévkönyv* (Issue 1, pp. 74–82). Dénes Natur Műhely.

Presentations in national and foreign conferences:

International conference:

Szőke, Z., Szemethy, D., Ujhegyi, N., Peer, G., Lakatos, I., Vörös-Láczó, E. & Szemethy, L. (2020): Mycotoxins in animal feed and the forage of wild herbivores: adverse physiological effects and implications of climate change. 17th International Scientific Days Online Conference, Gyöngyös. (presentation)

Keller N., Ujhegyi N., Biró Zs., Szemethy L., Farsang Zs. 2018. Haibat-use of the brown hare (*Lepus europaeus*) in an agricultural environment. In. Modern Aspects of

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Ujhegyi N., Biró Zs., Patkó L., Keller N., Tóth B., Kovács I., Szemethy L. 2015. Analysis of the impact on the brown hare (*Lepus europaeus*) population in Hungarian AES In. SCCS Hungary. (poster)

Ujhegyi N., Biró Zs., Patkó L., Keller N., Tóth B., Kovács I., Schally G., Csányi S., Szemethy L. 2015. Brown hare as an indicator species for Agri-environmental programs in Hungary. 27th ICCB- 4th ECCB, Montpellier. (poster)

Keller N., Ujhegyi N., Szemethy L. 2014. The connection between the agri-environmental schemes and the small game population in Hungary – preliminary study. In. "II. Sustainable development in the Carpathian Basin", Budapest. (presentation)

National conferences:

Ujhegyi N., Katona K., Keller N., Biró Zs. 2019. A mezei nyúl (*Lepus europaeus*) rejtőzködési és menekülési viselkedése emberi zavarás hatására agrártájban. XXI. Magyar Etológiai Társaság Konferenciája, Mátrafüred. (előadás)

Ujhegyi N., Biró Zs., Bókony V., Keller N., Patkó L., Szemethy L. 2018. A mezei nyúl (*Lepus europaeus*) demográfiai vizsgálata a mezőgazdasági tájhasználat függvényében. XX. Magyar Etológiai Társaság Konferenciája, Kolozsvár. (előadás)

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Keller N., Ujhegyi N., Biró Zs., Patkó L., Schally G., Szemethy L. 2016. Az Agrár-környezetgazdálkodási Támogatási Rendszer (AKG) vadgazdálkodási szempontú értékelése: a fejlesztés lehetőségei. X. Magyar Természetvédelmi Biológiai Konferencia, Mórahalom. (előadás)

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