# 14<sup>th</sup> European Vertebrate Management Conference

Ankaran, Slovenia 12—16 May 2025

# BOOK OF ABSTRACTS





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**Book of Abstracts** 

Editors: Boštjan Pokorny, Katarina Flajšman, Jens Jacob

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### PREFACE

The European Vertebrate Pest Management Conference (EVPMC) was first held in 1997 in York, UK. Since then, many successful conferences have taken place in various countries. Following the most recent edition, held in Florence, Italy, in 2023, it was decided that the 14<sup>th</sup> edition would be organized in Slovenia, in May 2025. This time, however, the acronym omits one letter—P for *pest*—following a general agreement to avoid potential stigmatization of various vertebrate species and to make the event more inclusive. Consequently, even more than in the past, the conference—now abbreviated as EVMC 2025—serves as a platform for presenting recent findings on vertebrate ecology, the ecological role and value of different species, hunting, behaviour, intra- and interspecific interactions, as well as human–wildlife coexistence, conflict, and population management.

In this context, the number of emerging topics and new scientific insights presented at the conference—and compiled in this Book of Abstracts—continues to grow. More than 200 participants from around the world attend the event in person and have the opportunity to read the abstracts of **167 contributions, which include 7 plenary talks, 88 oral presentations, and 72 posters**. These contributions are the outcome of the collaboration of an impressive variety of **806 co-authors from 45 countries**, including 28 countries with first authorship. The authors represent a broad geographical range, with contributions from 32 European countries and 13 countries across all other continents.

The contributions focus on the newest and most compelling topics, spanning a wide range of disciplines and taxa, including fish, amphibians, reptiles, birds, small mammals, lagomorphs, ungulates, mesocarnivores, and large predators. Several engaging symposia cover the following topics: Ecology, physiology and behaviour; Crops and urban systems; Human-animal conflicts and social dimension; Population monitoring and management; (Invasive) alien vertebrates; New tools and methods; Taxonomy and genetics; Health, zoonotic pathogens and parasites; and Rodenticide resistance and environmental monitoring.

We gratefully acknowledge the support of various institutions and companies that contributed to organizing the conference: the Ministry of Agriculture, Forestry and Food of the Republic of Slovenia, the Municipality of Ankaran, the Municipality of Velenje, as well as numerous sponsors and exhibitors: Ecotone GPS Telemetry, Pig Brig Trap Systems, DivjaLabs #BiodiversityInSight, the Botstiber Institute for Wildlife Fertility Control, International Pest Control, Unichem, MegaTel, Heslinga Traps, and Palma. We also extend our thanks to any new sponsor who may have joined in supporting the event after this Book of Abstracts went to press.

Finally, we would like to express our sincere gratitude to all partners and colleagues who have contributed—both visibly and behind the scenes—to the organization of EVMC 2025. Our thanks go to the plenary speakers, symposia chairs, all presenters, and to our colleagues from the Local Organizing Committee, who took responsibility not only for individual parts of the event but also for ensuring its overall success. Thank you for making EVMC 2025 a smooth, coherent, and truly memorable experience.

Thank you all for coming to Ankaran. We hope this event will be a memorable experience for you!

#### Boštjan Pokorny and Jens Jacob,

on behalf of the Organizing Committee and the International Steering Committee



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# **PLENARY TALKS**



### PLENARY TALK: Taxonomy

### Facing permanent taxonomic change

Kryštufek, Boris<sup>1,2\*</sup>

<sup>1</sup> Slovenian Museum of Natural History, Vertebrate Department, Ljubljana, Slovenia

<sup>2</sup> Science and Research Centre Koper, Mediterranean Institute for Environmental Studies, Koper, Slovenia

\* bkrystufek@pms-lj.si

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Four major taxonomic revisions published between 1982 and 2020 brought the total number of recognized mammalian species from the initial 4,629 species to the current 6,554 species, i.e. an astonishing increase of 57% in merely 38 years. Closer look at these lists shows that the estimate in each next edition is not merely the number in the previous issue with the addition of new discoveries in the interim period. Frequently, species are split through gain of new evidence which is not surprising given that taxonomic decisions are hypotheses rather than the reality itself. It comes more as a surprise that two globally used lists from 2020 reported widely different estimates: 5,899 species in the IUCN Red list versus the already quoted 6,554 species in the Checklist of the Mammals of the World. To make confusion even worse, experts involved in zoological systematics in general and species delimitation in particular take quite a variety of positions about the defining properties of a species. Over thirty species concepts are currently in circulation, each defining the species in at least slightly different ways. This may come as a surprise given that species are believed to be one of the fundamental units of biological organization underpinning every discipline of biology. Indeed, species lists have a wide range of users who have little time, interest or knowledge to interpret intelligently the results of taxonomic labour. The instability of taxonomic names is therefore perplexing to them, even more so when such changes are seemingly unnecessary.

Following de Queiroz, I argue that the source of the main problem with the species concept is in the confusion of two levels, the conceptual and operational one. Conceptually, species is a separately evolving metapopulation lineage. Such lineages are delimited by operational criteria (molecular, morphological, ecological etc.) which are currently referred to as species concepts. Two diverging lineages can be objectively ranked as distinct species only if they are sympatric. Sympatry allows an observer to safely conclude whether they are genetically (reproductively) incompatible but ecologically compatible. In other words, such lineages can co-occur because they can displace ecological niches on the one hand and maintain their genetic identity on the other hand. Disputes over taxonomic solutions invariably concern allopatric populations. Because allopatric entities do not interact in nature, uncertainties over their taxonomic status will continue in the future.

By using species lists of European mammals published since 1910, I will show that taxonomic change was far from being steady but instead showed at least one major twist in the mid-20<sup>th</sup> century. The overall effect of this change on our perception of mammalian diversity was strongly scale dependent. While it was insignificant at local scale, it heavily distorted the pattern at large scale.



### PLENARY TALK: Human-animal conflicts and social dimension

#### Human-crow conflicts from Europe to Africa

Kövér, László<sup>1\*</sup>

- <sup>1</sup> University of Debrecen, Department of Nature Conservation Zoology and Game Management, Debrecen, Hungary
- \* koverl@agr.unideb.hu

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The family of Corvidae (crows, choughs, jackdaws, jays, magpies, nutcrackers, ravens, rooks, treepies) is one of the most widespread bird groups globally. Corvids are highly adaptable and appear in a wide range of habitats: through forests, to the mountains, across open fields, as well as in urban environments. Their exceptional intelligence and versatility allow them to survive and succeed in such diverse conditions. From a human perspective, crows could be evaluated in different ways, depending on cultural context, judgments related to agriculture, nature conservation, and wildlife management, not to mention the general human attitude. We can mention several pest species that are being controlled or reduced in various ways. But there are also some endangered species as well. For instance, the Hawaiian crow (Corvus hawaiiensis) has already gone extinct in the wild, and huge amounts of money are being spent in attempts to save it. On the contrary, we hear the most and most often about species considered problematic/conflict, especially from urban environments. Nearly a quarter of Corvidae (30 species) regularly occur in urban environments. Crows have colonized and spread across many European cities in recent decades. They are often considered pests due to their noise, litter-spilling, predation on urban bird species, and aggression toward humans and domestic animals. Consequently, the control and management of crow populations have become necessary in many cases. This is the purpose of urban wildlife management, which is aimed at managing wildlife species occurring in cities. Its tasks include conducting various types of research, resolving conflicts between humans and animals, and educating people about urban species and how to coexist with them. During the presentation, the audience will get a general overview of human-crow conflicts, primarily in urban environments, and two specific case studies (from Europe and Africa) will also be presented.



### PLENARY TALK: New tools and methods

# Genetic insights for advanced conservation and management of large carnivores

Fedorca, Ancuta<sup>1\*</sup>

<sup>1</sup> National Institute for Research and Development in Forestry "Marin Dracea", Brasov, Romania

\* ancutacotovelea@yahoo.com

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Effective management of large carnivores in the Romanian Carpathians hinges on a robust understanding of their genetic structure, diversity, connectivity, and human-wildlife conflict. The Carpathians host some of Europe's most significant populations of these apex predators, making it essential to balance conservation priorities with strategies to mitigate human-wildlife conflicts at unprecedented levels. Genetic insights, including the broad uptake and use of genetic diversity for species assessments, are crucial in guiding management efforts. These insights, supported by scientific knowledge that underpins biodiversity protection, help monitor population size, gene flow, and genetic health, identify distinct subpopulations, and assess the impacts of habitat fragmentation. Digitalization in conservation enhances these efforts by enabling efficient data management, accurate analysis, streamlined research, and actionable reporting to support biodiversity protection under international agreements. This presentation reflects on the application of genetic research in the Carpathians and its implications for vertebrate management, focusing on advancing species conservation, promoting population connectivity, and fostering sustainable coexistence with local communities. By addressing ecological and socio-economic challenges, these efforts aim to secure the long-term survival of iconic carnivore species.



#### PLENARY TALK: Population monitoring and management

# Hunting and management of chronic wasting disease in cervids in North America and Europe

Mysterud, Atle<sup>1\*</sup>

<sup>1</sup> University of Oslo, Department of Biosciences, Centre for Ecological and Evolutionary Synthesis, Oslo, Norway \* atle.mysterud@ibv.uio.no

#### DOI: 10.20315/evmc.2025.004

Chronic wasting disease (CWD) is a contagious prion disease of cervids, and groups with diseases like bovine spongiform encephalopathy in cattle, scrapie in sheep, and Creutzfeldt–Jakob disease in humans. CWD was first diagnosed in deer in 1967 in Colorado, USA, and has spread to 35 states in USA and 4 provinces of Canada infecting mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and moose (*Alces alces*). The first detected case of CWD in Europe was in 2016 in a wild reindeer (*Rangifer tarandus*) in Norway. With onset of surveillance in Europe, a novel type of sporadic CWD, termed sCWD, was detected in moose and red deer (*Cervus elaphus*), but is not contagious and hence does not require hunting management. Prion diseases are invariably fatal, and there are no vaccines or treatments available. Contagious CWD has a long incubation period, where infected animals shed prions into the environment. Prevalence has reached high levels and led to deer population declines in North America. Hunting or culling is the main way to control CWD. Overall high harvest rate can limit CWD by shortening the infectious period and through other mechanisms.

Here, I review the main hunting strategies as part of CWD management: (i) depopulation, (ii) spatially targeted harvesting (around CWD cases), and (iii) male-biased harvesting. Other strategies have limited efficiency (e.g. targeting clinical suspects) or limited applicability (e.g. capture-test-and-cull), while the effect of density reduction per see remains uncertain. The success of each strategy depends on the species (level of site fidelity), habitat (open, forest), and population demarcation (open, closed). Achieving high harvest rates with recreational hunters only is challenging, and frequently requires the use of marksmen. Hence, success in limiting CWD strongly depends on the level of governmental involvement and public acceptance or resistance. I discuss the dynamic relationship between harvest rate to limit CWD and surveillance, and highlight the importance of pre-emptive culling, i.e., increased harvest before the first detection.



### PLENARY TALK: Population monitoring and management

# Challenges for ungulates in the era of the alpha generation

#### Šprem, Nikica<sup>1\*</sup>

- <sup>1</sup> University of Zagreb, Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Zagreb, Croatia
- \* nsprem@agr.hr

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The relationship between humans and ungulates is closely interwoven, especially as there are around 740 million inhabitants and around 25 million ungulates on the European continent. Europe is a largely human-dominated landscape, and the importance of ungulates is generally based on ecological, cultural, and economic reasons. Especially now, in the age of the alpha generation, the best-equipped and most technologically adept generation ever, where artificial intelligence is playing an increasingly important role, the greatest challenges for the coexistence of humans and ungulates are emerging.

Ungulates tend to increase their geographical range and numbers and are therefore the most important game species of great hunting interest, mainly for their meat and trophies. Throughout history, and especially today, the relationship between ungulates and humans is at a turning point due to various human interventions. There are several key points, but in this talk the focus will only be on reintroduction, hybridization, non-native species, and hunting management.

Reintroduction is the process of translocation and releasing wild animals into areas where they were previously extinct or considered endangered. Unfortunately, these interventions were often carried out with farm animals of unknown genetic origin, which can lead to hybridization. Hybridization between individuals of different (sub)species is a major conservation concern and can lead to changes in genetic, phenotypic, and fitness traits. Non-native species are widely recognised as a major global threat to biodiversity, with several species being classified as invasive without scientific basis. With this in mind, the focus will be on case studies of "invasive" ungulate species where policy decisions have been made without scientific basis. Ultimately, the impact of seven million hunters and an annual hunting bag of seven million ungulates in Europe could lead to evolutionary change.



### PLENARY TALK: Health, zoonotic pathogens and parasites

# Barriers to optimize integrated One Health monitoring including wildlife

Vicente Baños, Joaquin<sup>1\*</sup>

<sup>1</sup> Universidad de Castilla-la-Mancha, Instituto de Investigación en Recursos Cinegéticos, Ciudad Real, Spain

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Wildlife, including game, are not immune to global changes such as: loss of biodiversity, changes in land use, the movement of people, animals and animal products, and the rapid spread of transboundary diseases throughout the world. Wildlife can be severely impacted or otherwise, become reservoirs of pathogens that threaten human and animal health (such as livestock). Accelerated changes in the environment and management itself, often reactive and without technical bases with a long-term vision, can affect the sustainability of non-informed management. In this context, One Health (one human, animal, and environmental health) is a collaborative, multi-sectoral and transdisciplinary approach that addresses this problem at local, regional, national, and global levels. This concept places the natural world and wildlife at the centre of attention.

In this presentation we will (i) address the essential role of wildlife management in the One Health approach for a global solution, (ii) as well as the need for science-based monitoring under the One Health approach, and (iii) the main barriers to optimize this approach. The position of the wildlife managers and related stakeholders is strategic and fundamental due to its relationship with the natural environment; and its potential to contribute to the monitoring of wildlife, the environment, and shared diseases. For instance, this last aspect is essential for the early warning of emerging diseases, as first-line observers in surveillance systems for pathogens in wildlife, and thus improving the possibilities of their effective control and acting at source. However, there is an urgent need of strengthening collaboration with the rest of the sectors involved, and a better understanding of the perception of One Health (social aspects) by wildlife managers. In turn, it is also essential to promote the visibility of wildlife management principles and its potential contribution to the One Health approach to society, improving public perception and support for wildlife conservation through responsible management. The challenge is not to disconnect the sustainability of wildlife and game management from its necessary contribution to a One Health solution.



### PLENARY TALK: Conservation genetics

# Mighty mice and hybridizing hares: what rodents and lagomorphs tell us about chromosomes, conservation and climate change

Hauffe, Heidi C.<sup>1,2\*</sup>

<sup>1</sup> Research and Innovation Centre, Fondazione Edmund Mach, Conservation Genomics Research Unit and Platform for Animal, Environmental and Antique DNA, San Michele all'Adige, Italy

<sup>2</sup> National Biodiversity Future Center (NBFC), Palermo, Italy

\* heidi.hauffe@fmach.it

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Although there are dozens of species of rodents and lagomorphs in Europe, knowledge of their biology, ecology and genetic biodiversity is heterogeneous and heavily biased towards their traditional classification as laboratory models, agricultural pests, harbingers of disease or game species. While some of these taxa have been instrumental to our understanding of speciation and vector-borne zoonoses, in this brief (and almost certainly biased review), I would also like to present some of the more recent and innovative research in fields often reserved for larger and perhaps more 'charismatic' mammals. At the same time, it should be noted that almost all rodent/lagomorph species, predominantly vegetarian and serving as food sources for the higher trophic levels of most terrestrial ecosystems, are experiencing (severe) decline in numbers, often due to changes in agricultural practices and climate warming. The perception that rodents and lagomorphs are by definition common, widespread, and flourishing also means that almost 20 threatened species (and many more at a local level) are falling under the radar. Restocking and reintroductions are now considered acceptable actions even for these smaller mammals, and can be highly successful, although post-monitoring needs improvement. Such examples make interesting models in themselves for investigating the impact of these management interventions, given that understanding how to halt the decline of these important, fascinating yet unappreciated animals should be considered imperative to biodiversity conservation.



### PLENARY TALK: Ecology, physiology and behaviour

# What you always wanted to know about African mole-rats but never dared to ask

Begall, Sabine<sup>1\*</sup>

<sup>1</sup> University of Duisburg-Essen, Department of General Zoology, Essen, Germany

\* sabine.begall@uni-due.de

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African mole-rats (Bathyergidae) are strictly subterranean rodents spending most of their lives in selfconstructed burrow systems that they rarely leave. All bathyergids display morphological adaptations to life underground: cylindrical bodies, short extremities, prominent incisors or elongated nails as digging tools, to name just a few. Most research in this group has been focused on the naked mole-rat (*Heterocephalus glaber*) and their hairy cousins of the genus *Fukomys*. Various aspects of their sensory biology (especially hearing, vision, and magnetoreception) have been studied in detail. The animals are highly social, and reproduction is restricted to one breeding pair in each family. As the offspring stay for a considerable time with their parents, families consist of multiple generations which often have been referred to as "eusocial". Under laboratory conditions, mole-rats have a remarkably long maximum life-span with breeders living significantly longer than non-breeders. Reports about cancer incidence are close to zero, and at least naked mole-rats and Ansell's mole-rats (*Fukomys anselli*) are resistant to skin cancer after treatment with a strong mutagen.





# ORAL PRESENTATIONS



### How does hunting affect social contacts in wild boar populations?

Podgórski, Tomasz<sup>1,2\*</sup>; Fattorini, Niccolò<sup>3</sup>; Augustsson, Evelina<sup>5</sup>; Baubet, Eric<sup>6</sup>; Cagnacci, Francesca<sup>7</sup>; Fiderer, Christian<sup>8,9</sup>; Fischer, Claude<sup>10</sup>; Heurich, Marco<sup>8,9,11</sup>; Ježek, Miloš<sup>2</sup>; Kjellander, Petter<sup>5</sup>; Klamm, Alis<sup>12</sup>; Licoppe, Alain<sup>13</sup>; Morelle, Kevin<sup>14</sup>; Náhlik, András<sup>15,16</sup>; Nivois, Eveline<sup>6</sup>; Olejarz, Astrid<sup>2</sup>; Said, Sonia<sup>6</sup>; Suter, Stefan<sup>17</sup>; Tari, Tamás<sup>16</sup>; Vicente Baños, Joaquin<sup>18</sup>; Focardi, Stefano<sup>19</sup>

<sup>1</sup> Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland

<sup>2</sup> Czech University of Life Sciences, Faculty of Forestry and Wood Sciences, Prague, Czech Republic

<sup>3</sup> University of Siena, Department of Life Sciences, Italy

<sup>4</sup> National Biodiversity Future Center, Palermo, Italy

<sup>5</sup> Swedish University of Agricultural Sciences, Grimsö Wildlife Research Station, Riddarhyttan, Sweden

<sup>6</sup> French Office for Biodiversity (OFB), Birieux, France

<sup>7</sup> Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

<sup>8</sup> Bavarian Forest National Park, Grafenau, Germany

<sup>9</sup> University of Freiburg, Faculty of Environment and Natural Resources, Freiburg, Germany

<sup>10</sup> University of Applied Sciences of Western Switzerland, Department of Nature Management, Jussy, Switzerland

<sup>11</sup> Inland Norway University of Applied Sciences, Department of Forestry and Wildlife Management, Elverum, Norway

<sup>12</sup> Hainich National Park Administration, Department of Nature Conservation and Research, Bad Langensalza, Germany

<sup>13</sup> Service Public de Wallonie, Natural and Agricultural Environmental Studies Department, Gembloux, Belgium

<sup>14</sup> Max Planck Institute of Animal Behaviour, Department of Migration, Radolfzell, Germany

<sup>15</sup> Sapientia Hungarian University of Transylvania, Faculty of Life Sciences and Sports, Sfântu Gheorghe, Romania

<sup>16</sup> University of Sopron, Institute of Wildlife Biology and Management, Sopron, Hungary

<sup>17</sup> Zürich University of Applied Sciences (ZHAW), Wädenswil, Switzerland

<sup>18</sup> University of Castilla-La Mancha, Research Institute of Hunting Resources (IREC), Ciudad Real, Spain

<sup>19</sup> Istituto dei Sistemi Complessi Sezione di Sesto Fiorentino, Florence, Italy

\* podgorski@fld.czu.cz

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Variation in animal social interactions emerges from individual responses to social and physical environments and plays a key role in shaping pathogen transmission, gene flow, and information transfer. Although hunting can induce changes in contact patterns through disruption of the social environment, the non-consumptive effects of hunting have not received much attention compared to demographic effects of harvest. Therefore, we examined the effects of hunting activities on contact rates in wild boar, a species particularly exposed to social disruptions owing to its high sociality and intense management. Using GPS-telemetry data from 21 populations across Europe (435 unique dyads) we analysed how hunting activities impact social contacts within and between wild boar groups. Additionally, we tested the effects of covariates potentially shaping contact heterogeneity (sex, seasonality, spatial proximity). We found that drive hunts, but not individual hunts, lowered contact rates within groups while contacts between members of different groups were not affected by the hunting methods. The frequency of between-group contacts was mainly shaped by a positive relationship with spatial proximity. Between-group contacts were predominantly maintained by males which is consistent with the matrilineal social system of wild boar. Contact rates varied seasonally within groups and were relatively even throughout the year between groups. Our study showcases how disturbance caused by hunting affects contact rates in a group-living species. Along with other (a)biotic drivers, hunting modality plays a significant role in shaping intra-group, but not inter-group, contacts. This novel result helps understanding the anthropogenic drivers of the intraspecific social contacts, with critical implications for individual fitness, population processes and wildlife management when species with high epidemiological relevance, such as wild boar, are involved.



# Sleep in the wild: the importance of individual effects and environmental conditions on sleep behaviour in wild boar

Mortlock, Euan<sup>2</sup>; Silovský, Václav<sup>1</sup>; Guldenpfennig, Justine<sup>1</sup>; Faltusová, Monika<sup>1</sup>; Olejarz, Astrid<sup>1</sup>; Borger, Luca<sup>3</sup>; Ježek, Miloš<sup>1\*</sup>; Jennings, Domhnall<sup>2</sup>; Capellini, Isabella<sup>2</sup>

<sup>1</sup> Czech University of Life Sciences Prague, Department of Game Management and Wildlife Biology, Prague, Czech Republic

<sup>2</sup> Queen's University Belfast, School of Biological Sciences, Belfast, UK

<sup>3</sup> Swansea University, Department of Biosciences, Swansea, UK

\* jezekm@fld.czu.cz

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Sleep serves vital physiological functions, yet how sleep in wild animals is influenced by environmental conditions is poorly understood. Here we use high-resolution biologgers to investigate sleep in wild animals over ecologically relevant time scales and quantify variability between individuals under changing conditions. We developed a robust classification for accelerometer data and measured multiple dimensions of sleep in wild boar (*Sus scrofa*) over an annual cycle. In support of the hypothesis that environmental conditions determine thermoregulatory challenges, which regulate sleep, we show that sleep quantity, efficiency and quality are reduced on warmer days, sleep is less fragmented in longer and more humid days, while greater snow cover and rainfall promote sleep quality. Importantly, this longest and most detailed analysis of sleep in wild animals to date reveals large inter- and intraindividual variation. Specifically, short-sleepers sleep up to 46% less than long-sleepers but do not compensate for their short sleep through greater plasticity or quality, suggesting they may pay higher costs of sleep deprivation. Given the major role of sleep in health, our results suggest that global warming and the associated increase in extreme climatic events are likely to negatively impact sleep, and consequently health, in wildlife, particularly in nocturnal animals.



# Evidence of density dependence on wild boar reproduction in Mediterranean populations

Colomer, Joana<sup>1,2,3\*</sup>; Rodríguez-Teijeiro, José Domingo<sup>2,3</sup>; Rosell, Carme<sup>1,2</sup>; Massei, Giovanna<sup>4,5</sup>

<sup>1</sup> Minuartia, Barcelona, Spain

- <sup>2</sup> University of Barcelona, Faculty of Biology, Department of Evolutionary Biology, Ecology and Environmental Sciences, Barcelona, Spain
- <sup>3</sup> University of Barcelona, Institut de Recerca de la Biodiversitat, Barcelona, Spain
- <sup>4</sup> Botstiber Institute for Wildlife Fertility Control Europe, York, UK
- <sup>5</sup> University of York, Department of Environment and Geography, York, UK
- \* jcolomer@minuartia.com

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Understanding factors affecting the reproductive dynamics of wild boar populations is essential for developing effective population management. Previous studies have demonstrated density dependence in wild boar population growth but the mechanisms driving this relationship remain poorly understood. This study examines key reproductive parameters—ovulation rate, litter size, intrauterine mortality, and breeding probability—and their relationship with density across five Mediterranean sites over three years (2021-2023), using data from 575 female wild boar. Generalized linear mixed models (GLMMs) assessed the effects of population density on reproductive parameters, while structural equation models (SEMs) explored density-dependent mechanisms. A long-term dataset spanning 23 years was used to further explore the relationship between density and female body mass.

Results show that higher densities are associated with lower breeding probability, while ovulation rate, litter size, and intrauterine mortality were unaffected, suggesting that the primary reproductive adjustment to varying population densities lies in whether or not a wild boar female becomes pregnant, rather than the number of offspring produced. Female body mass influenced all reproductive parameters except intrauterine mortality. SEM analyses of the three years dataset did not reveal any effect of density on female body mass. Conversely, the long-term dataset demonstrated that higher population density is associated with reduced female body mass. This highlights the importance of long-term datasets in identifying processes that short-term studies might overlook. SEM also revealed a direct effect of density on breeding probability, independent of female body mass. This effect may reflect density-dependent changes in factors such as physiology, behaviour, or social structure, which ultimately influence reproduction. Our findings highlight the complex relationship between population density and reproductive success in wild boar and offer valuable information for understanding population dynamics and informing adaptive population management.



# How does climate change-induced drought affect European roe deer (*Capreolus capreolus*) fawn growth?

Flajšman, Katarina<sup>1\*</sup>; Pokorny, Boštjan<sup>1,2</sup>; Levanič, Tomislav<sup>1,3</sup>

<sup>1</sup> Slovenian Forestry Institute, Ljubljana, Slovenia

<sup>2</sup> Faculty of Environmental Protection, Velenje, Slovenia

<sup>3</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia

\* katarina.flajsman@gozdis.si

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The body mass of European roe deer (*Capreolus capreolus*) fawns reflects the condition/fitness of populations, but it can also reveal which environmental factors have significant influence on body growth and consequently on population dynamics. Therefore, body mass data and knowledge about environmental effects are important indicators for wildlife managers. When climate change-induced events are becoming more frequent, it is important to understand their impact to make wildlife management more efficient, sustainable, and up-to-date.

In this study, we analysed the body mass of roe deer fawns culled during the regular hunting period in Slovenia between September and December in the 14-year period 2010-2023 (in a very large sample set of 148,432 individuals: 67,161 males and 81,271 females). Data was collected from the Central Slovene Hunting Information System (OSLIS), in which several data on every culled individual are registered. We focused on interannual differences in body mass of fawns in four geographical regions: (i) Pre-Alpine, (ii) Pre-Pannonian, (iii) Sub-Mediterranean, and (iv) Karstic-Dinaric region.

On the country level, data showed that the body mass of fawns significantly increased from September to November in both sexes, while there was no significant increase between November and December. Interannual differences as well as differences among regions were statistically significant. Although interannual variability of body mass was generally low, we observed larger drops in years 2013, 2018, and 2022, which were all years with extreme hot and dry weather events during the summer months. In 2013, the dry period lasted from 11 June until 10 August, resulting in severe damage in agriculture due to drought. In 2018, the phenological development in summer was premature, fruits of some plants and wheat ripened up to one month earlier, and the drought was observed mostly in August. Similarly, the summer of 2022 was 2.8°C warmer and the precipitation reached only 59% of the normal (average) level. Multiple factors could affect the body mass of fawns. On average, female roe deer in Slovenia give birth in May, although there are slight regional differences. More importantly, roe deer is an income breeder, therefore individuals do not store body reserves for reproduction as they mostly rely on the availability of food resources during the rut period. Therefore, it is expected that interannual variability in food availability and/or quality due to weather-related effects would affect body mass and growth of fawns in a particular year. In accordance, our results showed that there are connections between body mass of fawns and the summer weather (drought), especially when comparing variability of body mass per year and region; however, we were neither able to statistically prove these observations nor connect fawn body mass with precipitation, mean temperature, and/or max July temperature.



# Can we open the forest for deer? Evaluating fencing as a method of forest protection against browsing

Borowski, Zbigniew<sup>1\*</sup>; Strękowska, Klaudia<sup>2</sup>; Borkowski, Jakub<sup>2</sup>

<sup>1</sup> Forest Research Institute, Department of Forest Ecology, Sękocin Stary, Poland

<sup>2</sup> University of Warmia and Mazury, Department of Forestry and Forest Ecology, Olsztyn, Poland

\* z.borowski@ibles.waw.pl

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Ungulate browsers, especially deer, when at high densities, may have adverse effects on their environment and are significant drivers of threatening forest biodiversity and ecosystem services. That is why foresters try to protect young forest stages against browsing and bark-stripping young trees by deer. In Poland, for the last 25 years, the most common method of forest protection against deer is fencing. Even though it is an expensive method, it also has a substantial ecological impact on the forest ecosystems, both in fenced and unfenced habitats. Logically, increasing the proportion of fenced forest habitats elevates deer pressure on unfenced areas. To better illustrate the scale of this phenomenon, it is good to know that the area of protected forest by fencing in the last 20 years in Poland increased fourfold in the previous 20 years, i.e. from 50,000 to 200,000 ha. What was essential, at the same time, was to observe an increase in populations of large predators (mainly wolves) and stabilization of red deer and roe deer populations in Polish forests.

With that in mind, the study aims to answer the question: are fences in forests necessary to protect forest plantations against deer browsing? We conducted a large-scale experiment in 19 forest districts (FD) in five different Poland regions to answer this question. In each FD, in half of their area, all new forest plantations were unfenced, whereas in the other part they were fenced as usual. At the same time, efforts were made to connect non-fenced areas to form a compact forest complex. In each FD, ten forest plantations were drawn on the fenced and unfenced parts for browsing monitoring. To analyse the browsing impact on the fenced part of the FD, the ten drawn forest plantations located on the fenced part remained unfenced. In the first year, significantly less pressure on young trees in unfenced parts of the FD was noted for all tree species. In contrast, however, similar deer pressure on palatable tree species (oak) on both fenced and unfenced parts was recorded in the second year, while browsing pressure on the remaining tree species was similarly lower in unfenced areas than in the first year. Interestingly, a significant relationship was observed between the size of the forest plantation (ranging from 0.2 to 8 ha) and the intensity of browsing pressure, but only in unfenced areas. The intensity of deer browsing increased by more than 40% with the increase in the plantation area. The results show that fencing forest plantations significantly impacts deer foraging and usually increases the pressure on young trees in unfenced areas. At the same time, browsing pressure on palatable tree species increases with increasing tree age and forest plantation area. We therefore recommend creating forest plantations with small areas (less than 1 ha) and limiting fencing to palatable tree species, such as oak, only.



# Variation in early life movements, protected areas and habitat use in juvenile Montagu's harriers (*Circus pygargus*) from central Italy

Monti, Flavio<sup>1\*</sup>; Alari, Guido Esposito<sup>2</sup>; Anselmi, Giuseppe<sup>3</sup>; Pezzo, Francesco<sup>4</sup>; Rizzo Pinna, Vincenzo<sup>2</sup>; Sforzi, Andrea<sup>2</sup>; Sammuri, Giampiero<sup>5</sup>

<sup>1</sup> Institute of Research on Terrestrial Ecosystems (IRET), National Research Council (CNR), Campus Ecotekne, Lecce, Italy

- <sup>2</sup> Maremma Natural History Museum, Grosseto, Italy
- <sup>3</sup> Alcedo APS, Grosseto, Italy
- <sup>4</sup> Italian Institute for Environmental Protection and Research (ISPRA), Ozzano dell'Emilia, Italy
- <sup>5</sup> Tuscan Archipelago National Park, Locality Enfola, Portoferraio, Italy
- \* flavio.monti@cnr.it

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Assessing individual differences and variability in movement patterns is essential to improve our understanding of the evolution and ontogeny of migratory strategies. In long-distance migratory species, fledged juveniles are extremely time-constrained in learning the essential skills for survival and preparing for migration, thus immediately facing a risky phase of their lives. Here we used high-resolution GPS/GSM transmitters fitted to juvenile Montagu's harriers (*Circus pygargus*) from central Italy to collect information on their movement ecology during early life stages, namely the post-fledging dependence period (PFDP) and the pre-migratory phase (PMP), until autumn migration. We were interested in investigating the spatiotemporal variation in home range, movement patterns, and habitat use during different stages of this period.

After fledging, individuals showed high variability, in both space and time, in home range size, daily distances covered, distance to nest and PFDP length. Residence time at the natal site significantly decreased, while the time interval between revisits in the natal area significantly increased as the PFDP progressed. During the PMP, explored areas and distance to nest (up to 320.8 km) varied among individuals, despite daily distances covered ( $27 \pm 40 \text{ km/day}$ ) and time allocation between traveling (60.7%) and foraging (39.3%) were similar across individuals. The PMP lasted 38 ± 14 days. Land cover composition of foraging locations was mostly represented by agricultural lands (~78.2%), though habitat use differed among individuals. More than 76% of such locations were located outside protected areas. Our novel individual-based tracking study improves previous knowledge based on field studies on the early life stages of the Montagu's harrier. High interindividual variability in movement patterns, broad-range exploratory movements and foraging locations "off-the-radar" of the protected area network make the application of standard conservation measures difficult, raising concerns about the long-term preservation of this vulnerable migratory species in Italy.



# Grazing reduced vegetation biomass and root nutrition related to plateau zokor creating mounds in summer on the Tibetan Plateau

Zhang, Zhiying<sup>1</sup>; Chu, Bin<sup>1\*</sup>; Hua, Limin<sup>1\*</sup>; Dong, Rui<sup>1</sup>; Dong, Kechi<sup>1</sup>; Cai, Xincheng<sup>1</sup>; Liu, Jize<sup>1</sup>; Gana, Ruixun<sup>1</sup>; Dong, Longming<sup>1</sup>; Zhang, Jing<sup>1</sup>; Sun, Wenqian<sup>1</sup>; Sun, Jian<sup>1</sup>; Niu, Yujie<sup>1,2\*</sup>

<sup>1</sup> Gansu Agricultural University, College of Grassland Science, Lanzhou, China

<sup>2</sup> University of Bayreuth, Department of Disturbance Ecology, Bayreuth, Germany

\* chub@gsau.edu.cn (B. Chu), hualm@gsau.edu.cn (L. Hua), Yujie.Niu@uni-bayreuth.de (Y. Niu)

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Plateau zokor (Eospalax baileyi) is an endemic, subterranean rodent native to the Tibetan Plateau. Its burrowing activity results in numerous bare mounds on the ground. These mounds interfere with plant community succession, affect carbon sequestration, reduce grazing areas, and intensify soil erosion, thus serving as key visual indicators of the zokor's ecological disturbances. Conventional views suggest that zokors primarily dig tunnels and then create mounds for mating and food storage in spring and autumn, respectively. Consequently, former studies have focused on mound creations only during these two periods. However, we found that plateau zokors also create mounds during the summer in the summer pastures. We designed experiments to investigate the environmental variables associated with this summer mound-creating phenomenon, and the results indicate that this behaviour is closely related to reduced vegetation biomass and nutrition in summer pastures. We further assessed the nutrition of plants and discovered that increasing mounding activity by zokors in summer corresponds with a decline in plant root quality, including reductions in crude protein, fats, and sugars, as well as fiber contents. Utilizing a random forest model, we found that the decrease in crude protein in plant roots is the principal factor influencing zokor mound creation in summer. These findings offer important insights for developing adaptive management strategies for alpine grasslands and assessing the environmental impact of the plateau zokor in alignment with the different grazing regimes.



# Spatial and temporal behaviour of commensal rodents in livestock farming

Huels, Florian<sup>1\*</sup>; Vanden Broecke, Bram<sup>2</sup>; Sluydts, Vincent<sup>2</sup>; Kirkpatrick, Lucinda<sup>2,3</sup>; Herrera Olivares, Ivan<sup>3</sup>; Vermeiren, Dries<sup>2</sup>; Ennen, Hendrik<sup>1</sup>; Leirs, Herwig<sup>2</sup>; Jacob, Jens<sup>1</sup>

<sup>1</sup> Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Rodent Research, Münster, Germany

- <sup>2</sup> University of Antwerp, Evolutionary Ecology Group, Wilrijk, Belgium
- <sup>3</sup> IoSA Tracking Internet of Small Animals, Antwerp, Belgium

\* florian.huels@julius-kuehn.de

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Rodents' spatial behaviour is a highly relevant feature in applied and basic research. Commensal rodents such as rats and house mice show diverse ecological and behavioural adaptations. Movement data can provide important insights into spatial and temporal activity patterns. In close proximity to humans, rodents can play a major role in pathogen transmission, especially in agricultural environments.

In this study, the spatial behaviour of rats and house mice on livestock farms and their potential role in pathogen transmission was investigated. Using a novel Bluetooth logger system, contact data and movement patterns of the rodents on the farms were recorded and identified. The results showed that there are differences among the commensal rodent species, i.e. black rat (*Rattus rattus*), Norway rat (Rattus norvegicus) and house mouse (Mus musculus), in temporal activity patterns, home range size, and movements between individual stable buildings. Rat species showed typical night activity whereas house mice were active throughout day and night. House mice travelled significantly longer distances while rats occupied larger home and core area sizes. The rodents used certain preferred areas and did almost never move between buildings. While movements between different stables were rare, movements within the buildings were frequently recorded and occasional excursions to the perimeter of neighbouring buildings occurred. The probability of direct contact with livestock and their feeding stations and thus a possible transmission of pathogens seems mostly related to the rodents' resident in a particular building. The contact data showed that rats avoid encounters with other individuals outside their core areas. The social network identified clusters of contacts among individuals where pathogen transfer is most likely. The practicability of Bluetooth proximity loggers for the study of small mammal spatiotemporal behaviour could be successfully demonstrated.

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### Range expansion during re-colonization: the advantage of being shy

Jánošíková, Radoslava<sup>1\*</sup>; Tulis, Filip<sup>1</sup>; Baláž, Ivan<sup>1</sup>; Eccard, Jana A.<sup>2</sup>; Mazza, Valeria<sup>2,3</sup>

<sup>1</sup> Constantine the Philosopher University, Department of Environmental Sciences, Nitra, Slovak Republic

<sup>2</sup> University of Potsdam, Institute for Biochemistry and Biology, Animal Ecology, Potsdam, Germany

<sup>3</sup> University of Tuscia, Department of Ecological and Biological Sciences, Viterbo, Italy

\* r.janosikova@gmail.com

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Pioneer individuals at the edge of an ongoing expansion face unique ecological and evolutionary challenges. It shapes their behaviour to be different from stable, long-established populations. This study investigated behavioural responses associated with risk-taking and exploratory tendencies. It was conducted on small mammals across different colonization stages using a standardized behavioural test in the field. We quantified exploration and boldness of striped field mice (*Apodemus agrarius*, n=95) from six subpopulations. The population is already established in Germany, and it is re-colonizing Slovakia. The study also included bank voles (*Myodes glareolus*, n=76) that shared the same habitats but were long-established at all sites. We hypothesized that behavioural traits, such as exploration and risk-taking, would differ. This would be highly expressed in the population at the expansion edge compared to conspecifics in long-established populations. Individuals facing novel environments could benefit from adaptive strategies that enhance their survival and dispersal.

Our findings revealed no significant difference in the measured behaviour between two populations of established bank voles. Striped field mice in the expanding populations were slower in exploring the test arena but showing comparable levels of risk taking as established populations. A slow and thorough exploration strategy might play an advantageous role in the expansion processes of small mammal populations. This could compensate for their reduced speed in resource discovery and acquisition compared to individuals in established populations.



### Beyond borders: expansion of striped field mouse in Slovakia

Košša, Jakub<sup>1\*</sup>; Tulis, Filip<sup>1</sup>; Baláž, Ivan<sup>1</sup>, Jánošíková, Radoslava<sup>1</sup>; Ambros, Michal<sup>2</sup>; Baláž, Michal<sup>3</sup>; Poláčiková, Zuzana<sup>4</sup>; Jureček, Rudolf<sup>5</sup>; Dudich, Alexander<sup>6</sup>; Stollmann, Andrej<sup>7</sup>; Ševčík, Michal<sup>1</sup>

- <sup>1</sup> Constantine the Philosopher University in Nitra, Faculty of Natural Sciences and Informatics, Department of Environmental Sciences, Nitra, Slovakia
- <sup>2</sup> ŠOP SR, Administration of CHKO Ponitrie, Nitra, Slovakia
- <sup>3</sup> Catholic University in Ružomberok, Faculty of Education, Ružomberok, Slovakia
- <sup>4</sup> Constantine the Philosopher University in Nitra, Faculty of Natural Sciences and Informatics, Department of Zoology and Anthropology, Nitra, Slovakia
- <sup>5</sup> ŠOP SR, Administration of CHKO Záhorie, Malacky, Slovakia
- <sup>6</sup> Nám. Sv. Trojice 15, Banská Štiavnica, Slovakia
- <sup>7</sup> Krivá 10, Hurbanovo, Slovakia
- \* jakub.kossa@ukf.sk

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Range expansion, as an extension of the normal colonisation process, is characterised by establishment and an increase in abundance. This study combines conventional methods with citizen science to investigate the occurrence of the striped field mouse (Apodemus agrarius; thereafter SFM) in Slovakia and its ongoing expansion. In 2024, fifty years after the first records of SFM's spreading in eastern Slovakia, the species expanded its range to approximately 18,600 km<sup>2</sup>, reflecting a remarkable 135% increase in occurrence compared to its initial distribution in 1974. We observed a dynamic increase in SFM's dominance following the colonization of new sites, with no significant seasonal effects on its abundance. This sustained rise in dominance signifies successful establishment and indicates that the "expansion front" is effectively overcoming environmental barriers, thereby facilitating further spreading into adjacent territories. Our findings suggest that the expansion process of native species like the SFM can mimic characteristics typically associated with invasions by alien species, raising important ecological implications regarding species interactions and community dynamics. Utilising citizen science significantly enhanced our data collection efforts, allowing for a more comprehensive understanding of SFM's distribution and abundance. This collaborative approach not only enriched our dataset but also engaged local communities in conservation efforts, demonstrating the value of citizen science in ecological research and species monitoring.

In conclusion, the striped field mouse's expansion serves as a compelling case study illustrating how native species can exhibit invasive-like behaviour concerning dominance and range increase. Our results emphasize the necessity for ongoing monitoring of small mammals and the development of effective management strategies to address potential ecological impacts arising from their spreading. Understanding these dynamics is crucial for biodiversity conservation and habitat management in the face of changing environmental conditions.

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## The effects of pair bond interruption on emotion in male mandarin voles and its neuromechanism

Shi, Jinxiao<sup>1</sup>; Zhao, Peiwen<sup>1</sup>; Cui, Shunping<sup>1</sup>; Li, Changxin<sup>1</sup>; Yang, Yang<sup>1</sup>; Yang, Yuying<sup>1</sup>; Jia, Rui<sup>1\*</sup>

<sup>1</sup> Shaanxi Normal University, College of Life Sciences, Xi'an, Shaanxi, China

\* jiarui@snnu.edu.cn

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Social bonds play a pivotal role throughout the lifespan of (social) animals including humans. Stable and long-lasting social bonds have positive effects on emotion, physiology, and behaviours. However, the neuromechanism of pair bond interruption on emotion is not clearly known. In this study, monogamous male mandarin voles (*Lasiopodomys mandarinus*) were used to study the neuromechanisms of the oxytocin (OT) system after pair bond interruption which likely induced emotion disorders.

The results showed that pair bond interruption can induce anxiety- and depression-like behaviours in mandarin voles. We find that pair bond interruption impaired maturation of dendritic spines in the NAccShell, increased neuron activity in the paraventricular nucleus (PVN), decreased OT-ir fiber density to NAccShell, inhibited neuron activity and OT receptor protein levels in the NAccShell. Exogenous administration of OT and OTR-A influenced behaviours, with OTR-A mimicking interruption effects and OT reversing them. In conclusion, the present study proved that pair bond interruption can induce emotion disorders in male mandarin voles and the OT system in the PVN-NAccShell neural circuit may play a key role to modulate behaviours. These conclusions enhance our understanding of the neuroendocrine mechanisms underlying emotional disturbances caused by pair bond interruption, and provide a theoretical basis for future research and treatment of emotional disturbances induced by pair bond interruption.



### Comparative study on the metabolic regulation patterns of skeletal muscles in rodents at different altitudes under hypoxic conditions

Li, Mengyang<sup>1</sup>; Wang, Zhenlong<sup>1\*</sup>

<sup>1</sup> Zhengzhou University, School of Life Sciences, Zhengzhou, China

\* wzl@zzu.edu.cn

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Skeletal muscle, a pivotal regulator of metabolic equilibrium, facilitates body movement through the contraction and relaxation of muscle fibers and is highly dependent on mitochondrial substrate oxidation for energy production. Hypoxic environment will lead to the reduction of oxidative capacity in muscle cells and insufficient energy supply, which will affect the physiological function and survival of mammals. However, the skeletal muscle of mammals living in hypoxia environment for a long time has formed obvious evolutionary characteristics of hypoxia adaptation in terms of morphology, structure and physiological function. Here, we carried out transcriptomics and quasi-targeted metabolomics analysis on skeletal muscle tissue of Qinghai vole (Neodon fuscus), Brandt's vole (Lasiopodomys brandtii) and Kunming mice (Mus musculus) after hypoxia (10% O<sub>2</sub>, 48 h) and normoxia treatment, thereby revealing the underlying molecular mechanisms. N. fuscus, native to high-altitude environments, tended to sustain energy supplies through enhanced fatty acid oxidation under lowoxygen conditions. Conversely, L. brandtii and M. musculus, acclimatized to middle- and low-altitude habitats, relied on aerobic oxidation and anaerobic glycolysis of glucose, respectively, for energy maintenance under hypoxic conditions. In addition to their differential metabolic preferences under hypoxic conditions, these three rodent species showed species-specific responses related to oxygen utilization, antioxidant defence mechanisms, and anti-inflammatory processes. This study provides insights into the metabolic response patterns of mammalian skeletal muscle under hypoxic conditions, thereby establishing a basis for future investigations on transcriptional-metabolic associations.



# Survival, habitat selection, and success of wildlife reinforcement: a case study of European brown hares in a grassland-dominated landscape

Henriot, Charles Pierre<sup>1\*</sup>; Bonenfant, Christophe<sup>2</sup>; Berger, Léopold<sup>1</sup>; Powolny, Thibaut<sup>3</sup>; Renaude, Régis<sup>3</sup>; Sage, Mickaël<sup>1</sup>

<sup>1</sup> Faune INNOV' R&D, Besançon, France

<sup>2</sup> Université Claude Bernard Lyon 1, Laboratoire de biométrie et biologie évolutive, Villeurbanne, France

<sup>3</sup> Fédération Départementale des Chasseurs du Doubs, Gonsans, France

\* charles.henriot@faune-innov.fr

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The anthropisation of environment and habitat fragmentation are major drivers of biodiversity loss, eroding the resilience and genetic diversity of local populations, making wildlife reinforcements increasingly necessary to ensure their conservation. However, the factors influencing the success of such operations, in particular the survival and habitat selection of released individuals, remain poorly documented for certain species and certain landscape types. Several studies have investigated the effects of European brown hare (*Lepus europaeus*) translocations. However, the survival and habitat selection behaviour of released hares, as well as the spatial and temporal impacts of reinforcements, remain poorly understood in grassland landscapes. Therefore, our study aims to characterise the impacts of hare reinforcements into grassland-dominated landscapes with low hare densities in terms of: (i) survival and causes of mortality of released individuals, (ii) their habitat selection, and (iii) the overall spatial and temporal impact of releases on local population dynamics.

A total of 103 hares from a professional breeding facility were released in two waves (51 in 2021 and 52 in 2022) in a mid-mountainous grassland-dominated landscape (2000 ha, Jura Mountains, France) with a low hare density. Movements and survival were monitored using GPS collars and the carcasses were examined in a veterinary lab to determine the cause of death. Local population density estimates were conducted before, during, and after releases using point transect sampling in and outside the reinforcement area (63 points in 10,000 ha large area).

Overall survival rates were low, primarily due to predation, roadkill, and diseases although the distribution of causes varied over time. At the landscape level, released hares favoured closed habitats (i.e., forest and hedges), while avoiding built areas and roads. Within their home range, hares selected closed habitats during the day (inactive period) and open habitats (i.e., grasslands and winter cereals) at night (active period). Bayesian spatiotemporal generalized linear mixed models indicated that reinforcements had little short-term overall impact on local hare population dynamics. The spatial impact of releases was restricted, while the observed densities increased post-reinforcement. These findings provide crucial insights to optimize population reinforcement efforts by identifying preferred habitats and key mortality factors for released hares. This information not only enables future releases to be targeted in more favourable environmental conditions—ultimately increasing survival rates and supporting long-term stability of reinforced populations—but also enhances our understanding of ecological dynamics and habitat requirements of wild hare populations.



### Overlooked giants: edible dormouse as a keystone species of temperate deciduous forests?

Jerina, Klemen<sup>1\*</sup>; Bordjan, Dejan<sup>1</sup>; Kryštufek, Boris<sup>2,3</sup>; Ponikvar, Lovrenc<sup>1</sup>

<sup>1</sup> University of Ljubljana, Biotechnical Faculty, Department of Forestry, Ljubljana, Slovenia

<sup>2</sup> Slovenian Museum of Natural History, Vertebrate Department, Ljubljana, Slovenia

<sup>3</sup> Science and Research Centre Koper, Mediterranean Institute for Environmental Studies, Koper, Slovenia

\* klemen.jerina@bf.uni-lj.si

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Deciduous forests that produce fat-rich mast (oak, chestnut, especially beech) are prime habitats for edible dormouse (Glis glis) within its global distribution range—Europe and Asia Minor. In such forests, dormouse biomass can exceed the combined biomass of all ungulate species. The dormouse experiences substantial predation pressure and serves as a food (either occasionally or as a seasonally significant resource) for numerous omnivorous and carnivorous species of mammals, birds, and reptiles, in size spanning from least weasel to brown bear. Energetically, dormouse may represent an important link between fat-rich seeds and higher trophic levels, thus driving forest ecosystems; due to its multiple ecological roles, it may be considered a keystone species. The majority of contemporary dormouse research focuses on its activity, hibernation, synchronization of reproduction and activity with mast years, and its exposure to predators. However, most studies are conducted outside karst regions, which are among the best habitats for this species. In non-karst areas, dormice often use tree cavities, bird nesting boxes, underground hibernacula, and buildings as daily and winter dens. In contrast, in karst regions, they typically rely on the numerous caves and other underground caverns for both winter hibernation and daily rest. Dormice are particularly vulnerable to predation during their active periods, e.g. while foraging in tree canopies. A much narrower range of predators can hunt dormice during their inactive periods, which they typically spend underground in karst caverns. The entrances to these caverns are usually narrow, allowing dormice to pass while excluding larger predators. Dormice daily concentrate at these entrances, with many individuals sharing the same cavern. Their activity is predictable, and on the ground, they are less agile compared to their movements in the canopy. As a result, these entry points may serve as effective hunting sites for predators, a hypothesis that remains unexplored.

The aim of this study was to analyse the seasonal and interannual dynamics of dormouse activity and the species composition of predators at the entrances to underground caverns. We continuously monitored the entrances to ten dormouse caverns using black infrared camera traps over two years—good and poor beech mast year. During the poor mast year, we recorded 250 dormouse entries/exits (compared to over tenfold more in the good mast year) and 480 instances of eight predator species lingering near the entrances: stone/pine marten (266), wildcat (68), red fox (67), domestic cat (45), badger (19), brown bear (17), and wild boar (4). Predators frequently displayed signs of vigilance and appeared to monitor the entrances, suggesting they were hunting dormice and synchronizing their activity near the shelters with that of the dormice. Our research suggests that dormouse cavern entrances are an efficient hunting site for major medium and large mammalian species of predators of dormouse. At the same time, it indicates how dormouse optimize its fitness and survival by alternating between the use of safe underground caverns and foraging in tree canopy.



### What do wolves eat? Insights into diet variation of an apex predator in Central and Southern Europe using faecal DNA metabarcoding

De Barba, Marta<sup>1,2\*</sup>; Konec, Marjeta<sup>1,2</sup>; Boljte, Barbara<sup>2</sup>; Pazhenkova, Elena<sup>1,2</sup>; Hrga, Nuša<sup>2</sup>; Majić Skrbinšek, Aleksandra<sup>1,2</sup>; Simčič, Gregor<sup>2</sup>; Brenner, Wiebke<sup>3</sup>; Kozyra-Zyskowska, Katarzyna<sup>4</sup>; Sharma, Sandeep<sup>5</sup>; Malić Limari, Snježana<sup>6</sup>; Skrbinšek, Tomaž<sup>1,2</sup>

- <sup>2</sup> University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia
- <sup>3</sup> Rewilding Oder Delta e.V., Rothenklempenow, Germany
- <sup>4</sup> Praca Przyrodnika Sp. z o.o., Szczecin, Poland
- <sup>5</sup> German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany
- <sup>6</sup> WWF Adria, Zagreb, Croatia
- \* marta.debarba@gmail.com

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Trophic interactions, which encompass the diet of a species and its dynamics in natural systems, shape the distribution of species and their ecological niche, and influence population dynamics, viability, and responses to environmental changes as well as ecosystem function. Apex predators, such as grey wolf (*Canis lupus*), play critical roles in ecosystems, but their diets can be influenced by human activities, also exacerbating human-wildlife conflicts. Accurate knowledge of diet composition and feeding behaviour of apex predators is therefore instrumental for effective management and implementation of conflict mitigation measures for conservation.

Using a DNA metabarcoding approach we investigated the diet of wolves in three European regions with differing socio-economic and environmental conditions: Alps in Slovenia, the Dinaric region in Croatia, and the Oder Delta across Germany and Poland. Faecal samples (n = 70, 60, and 78, respectively) collected in 2019-2024 were analysed using a metabarcode marker to identify vertebrate/mammalian prey. Results revealed clear spatial patterns in diet composition driven by prey availability. Wild ungulates, particularly red deer (*Cervus elaphus*) and European roe deer (*Capreolus capreolus*), emerged as primary prey across all regions, reflecting known preference by wolves when available. Wild boar (*Sus scrofa*) was also frequently detected, especially in Croatia, in areas where the preferred natural prey abundance declined. Occurrence of domestic animals (e.g. cattle, goat, sheep) in the samples was generally low, but increased with higher incidence of human presence and activities. In addition, the same DNA extracts processed for diet analysis were also used for individual genotyping with potential for deciphering individual feeding habits, but also providing an example of cost-effective use of faecal samples typically collected in genetic monitoring programs for multiple purposes.

This study provides high-resolution dietary data and a deeper understanding of the ecological role of wolves important for informing management in different parts of Europe. It also demonstrates the power of DNA metabarcoding for efficient non-invasive diet assessment which is crucial for enabling large scale ecological studies, such as to explore spatiotemporal variation in diet through environmental gradients or as a consequence of anthropogenic activities and global changes, ultimately enhancing future conservation efforts.



<sup>&</sup>lt;sup>1</sup> DivjaLabs Ltd., Ljubljana, Slovenia

## Population dynamics as a mediator of improved wild animal welfare from the use of wildlife contraception

Eckerström Liedholm, Simon<sup>1\*</sup>; Hecht, Luke<sup>1</sup>; Elliott, Vittoria<sup>2</sup>

<sup>1</sup> Wild Animal Initiative, Minneapolis, USA

<sup>2</sup> National Museum of Natural History at the Smithsonian Institution, Washington DC, USA

\* simon.liedholm@wildanimalinitiative.org

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Populations close to their carrying capacity are often suppressed through mechanisms that are likely to affect welfare negatively, such as starvation, intraspecific competition, and disease. Although usually thought of only as a non-lethal approach to population control, wildlife contraception has the potential to improve wild animals' welfare above natural baselines, by averting negative consequences of high population density. Knowing under what conditions average welfare may be expected to increase in a population controlled through the use of wildlife contraception has potential practical implications for the management of wild animals. In general, positive welfare effects of wildlife contraceptives are more likely to occur when reproduction continues unabated and survival is reduced at high densities, and less likely when adults naturally limit immediate reproduction (defined as 'self-imposed fertility control') in favour of somatic maintenance and parental care. Furthermore, wildlife contraception is likely to have the largest positive impact on welfare where large declines in juvenile survival occur between growing and stable population states, and where these declines steepen as populations approach carrying capacity. Lastly, the welfare benefits to already managed populations of wild animals are likely larger when the current methods are expected to cause protracted deaths, such as from the use of rodenticides, compared to other common causes of death for those animals. We present a framework, based on these principles, for predicting how much a given species stands to benefit from wildlife contraception based on its life history strategy and other traits.



## Effects of temperature and precipitation on nesting of olive ridley turtles in western Bay of Bengal

Banerjee, Kakoli<sup>1\*</sup>; Paul, Rakesh<sup>1</sup>; Bindhani, Pramod Kumar<sup>1</sup>; Kurhe, Anil<sup>2</sup>

<sup>1</sup> Central University of Odisha, Department of Biodiversity and Conservation of Natural Resources, Sunabeda, NAD, Koraput, India

<sup>2</sup> Padmashri Vikhe Patil College, Department of Zoology, Pravaranagar, Ahmednagar, Maharashtra, India

\* banerjee.kakoli@yahoo.com

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Marine turtles inhabit tropical and temperate seas worldwide. Adult turtles of most species are commonly found in shallow coastal waters, bays, lagoons and estuaries, although some species also explore the open ocean. Rushikulya Beach and Gahirmatha Marine Sanctuary, known for their coastal environments, host the globally renowned olive ridley turtle nesting ground. Situated in the vicinity of Rushikulya River in Ganjam and Bhitarkanika Wildlife Sanctuary in Kendarapara, a protected zone, this area visualizes the maximum number of turtles nesting in the state and the country. The impact of climatic parameters was studied in this marine sanctuary in order to find out whether turtle nesting is dependent upon these two independent parameters. Similarly, the climatic data were procured from IMD, Bhubaneswar, and some online sources. The study showed that the trend of temperature over the years is decreasing, rainfall is more or less uniform with a rise in the recent decade, whereas the wind speed has a rising trend. Cyclonic data also shows that over the past two decades, there have been increased cyclones on the coast of Odisha. The study, however, shows that turtle nesting increased during 2016-2018 but again decreased during 2019-2020, which may be due to Cyclone Fani and also the erosion of the coastline. Considering future and ongoing natural phenomena, it is crucial to comprehensively understand, evaluate, and study shoreline changes and related processes to ensure the conservation of signature species and their habitats.



## Efficacy of anticoagulant rodenticides applied to Norway rats (*Rattus norvegicus*) in the sewer system of Berlin, Germany

Schlötelburg, Annika<sup>1\*</sup>; Meneghini, Dorina<sup>2</sup>; Fickel, Jörns<sup>2,3</sup>; Schmolz, Erik<sup>4</sup>; Geduhn, Anke<sup>1</sup>

<sup>1</sup> German Environment Agency, Department of Health Pests and their Control, Berlin, Germany

<sup>2</sup> Leibniz Institute for Zoo and Wildlife Research, Department of Evolutionary Genetics, Berlin, Germany

<sup>3</sup> University of Potsdam, Institute of Biochemistry and Biology, Potsdam, Germany

\* Annika.schloetelburg@uba.de

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When second generation anticoagulant rodenticides used to control rats in sewers come in contact with water, the active substances accumulate along the aquatic food chain. Residues of these substances have already been detected in fish and their predators, such as Eurasian otters (*Lutra lutra*), cormorants (*Phalacrocorax carbo*), and common merganser (*Mergus merganser*). Despite their potentially detrimental effects to the wider ecosystem, rodenticides are still used to control urban rats in sewage systems. However, some cities like Zurich (Switzerland) and Erfurt (Germany) are managing urban rats without using rodenticides in the sewers.

Against this background, we investigated the efficacy of chemical rat control in sewers of six study areas in Berlin. We determined with two non-invasive monitoring methods the degree of rat infestations in the sewage system and adjacent parks, backyards or waste disposal sites at three time points: before, after, and six months after rodenticide application in the sewers. Underground, we applied wildlife cameras and measured feeding of a non-toxic bait; aboveground, we counted rats with a thermal camera and conducted a feeding census. Furthermore, we caught 262 rats and used their muscle tissue for genetic analysis. For the latter, an array with 96 SNPs (single nucleotide polymorphisms) was established to determine individual genotypes using 89 samples from rats of different parts of Berlin. Comparison of genotype frequencies at sampling sites before and after the treatment will show whether local gene pools have changed in size and allele frequencies after rodenticide application, and if rat packs have recovered within six months through reproduction or migration. In addition, we studied if individuals from above and underground were reproductively connected or separated. The latter would question the efforts to control rats in sewers, as they pose a neglectable health threat if they only occur underground.

First results show that the chemical control in the sewers initially reduced the number of underground rats, but to a degree only that allowed the populations to recover within six months. The aboveground populations appeared to be largely unaffected by the chemical control in the sewers. These results and the insights into the ecology of urban rats will help to develop a new urban rat management plan in order to use rodenticides only where necessary and effective.

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<sup>&</sup>lt;sup>4</sup> German Environment Agency, Division IV Chemical Safety, Dessau-Roßlau, Germany

### Non-chemical rodent management in organic carrot cultivation – vole activity and economic assessment

Dürger, Joanna<sup>1</sup>; Trebus, Hans<sup>2\*</sup>; Karpinski, Isabella<sup>2</sup>; Leucker, Marlene<sup>3</sup>; Reising-Hein, Christian<sup>3</sup>; Jacob, Jens<sup>4</sup>; Fischer, Christina<sup>1</sup>

- <sup>1</sup> Anhalt University of Applied Sciences, Faunistics and Wildlife Conservation, Department of Agriculture, Ecotrophology, and Landscape Development, Bernburg, Germany
- <sup>2</sup> Julius Kühn-Institute, Institute for Strategies and Impact Assessment, Kleinmachnow, Germany
- <sup>3</sup> Chamber of Agriculture North Rhine-Westphalia, Plant Protection Service, Plant Protection in Vegetable Crops and Warning Service, Cologne-Auweiler, Germany
- <sup>4</sup> Julius Kühn-Institute, Institute for Epidemiology and Pathogen Diagnostics, Rodent Research, Münster, Germany
- \* hans.trebus@julius-kuehn.de

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Mass reproduction of common voles occurs every 3-5 years. Especially in vegetable production during this time, yield losses are substantial, and total loss can be reached. Damaged vegetables cause increased sorting effort and cannot be sold. In German (organic) cropping, the carrot is the most important vegetable crop with a total yield of almost 800,000 tonnes in 2023. The evaluation of vole occurrence/activity and the cost-effectiveness of non-chemical alternatives for rodent management in this segment is of great importance for farming, as the use of chemical rodenticides is prohibited. Here, common vole activity, damage and yield were assessed in organic carrot fields in North Rhine-Westphalia, Germany. Six fields were protected with a trench and at two fields no vole protection measures were introduced (experimental control). Activity indices were estimated in field edges and adjacent carrot fields from hair tubes or counts of active burrow entrances according to standard procedures to estimate common vole abundance. Vole damage and yield were determined on a fieldspecific basis. The economic efficiency of the tested measures was assessed with cost-benefit analysis. The direct costs of the management using trenches were compared with the financial losses due to yield reduction in fields without trenches. The resulting vole management cost-free performance is suitable as a benchmark for comparing the results of the field trials with secondary data-based scenario calculations.

First results indicate that during low population density of voles such as in the year 2024 there are no significant differences in vole activity in the field edges and the adjacent carrot fields. However, trenches may lose their effectiveness as a dispersal barrier for voles if they become overgrown or exposed to heavy rainfall. Further information will be collected in 2025 to contribute to the implementation of integrated plant protection in rodent management in organic farming.

This project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming (2819OE179, 2819OE180, 2819OE182).



## Biological control of the common vole (*Microtus arvalis*) in Castilla y León: an innovative approach with weasels (*Mustela nivalis*)

González-Simón, Paula<sup>1\*</sup>; Báscones-Reina, Miriam<sup>1</sup>; Hernández-Garavís, Lorena<sup>1</sup>; Garcés-Toledano, José Fernando<sup>1</sup>; Cuéllar-Basterrechea, Carlos<sup>1</sup>; Rodríguez-Moreno, Beatriz<sup>1</sup>; Blanca-Chana, Fernando<sup>1</sup>

<sup>1</sup> Group for the Rehabilitation of Native Fauna and its Habitat (GREFA), Majadahonda, Spain

\* paulagonzalez@grefa.org

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The common vole (*Microtus arvalis*) is a rodent that regularly causes significant damage to crops and public health issues in Castilla y León (Spain). As an alternative to chemical methods and other measures that can be harmful to biodiversity, a biological control project has been implemented based on the promotion of the natural predators of this rodent. This initiative has worked successfully in experimental projects with nest boxes for raptors. However, there are areas that can serve as reservoirs for the common vole where the vegetation is dense, limiting the effectiveness of predatory birds. Therefore, we started studying the potential of using weasels (*Mustela nivalis*) as complementary predators by installing shelter boxes for them in three different areas of Castilla y León. The purpose was to assess the suitability of the shelter's design for these mustelids and to monitor their behaviour in order to evaluate their potential in pest control.

Preliminary results show a positive response of the weasels to the shelter-boxes and an increase in their activity in areas with high vole density. However, further studies are needed to optimize the design of the shelters and to assess the feasibility of releasing specimens from captive breeding programs. This project represents a significant advance in integrated pest management and opens new perspectives for the conservation of biodiversity in agroecosystems.



## Fertility effects of a candidate gene for sustainable control of invasive house mice (*Mus musculus*)

Friry, Salomé<sup>1\*</sup>; Lindholm, Anna K.<sup>1</sup>; Manser, Andri<sup>1</sup>

<sup>1</sup> University of Zürich, Department of Evolutionary Biology and Environmental Studies, Zürich, Switzerland \* salome.friry@uzh.ch

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There are limited means of sustainably controlling invasive house mice (Mus musculus) populations, especially on islands where poison dissemination threatens endemic species and raises welfare concerns. Currently, we are investigating whether the *t*-haplotype, a naturally occurring meiotic driver in house mice, could be used as a more humane and specific alternative for pest control. In t-heterozygous males, the t-haplotype gains a transmission advantage by "sabotaging" wild-type sperm, whilst t-homozygous males are sterile. Therefore, control might be achieved by elevating the frequency of sterile males through the release and reproduction of t-carrying males into target populations. To accurately assess the impact of t-carrier releases, we measured the t-haplotype's fertility effects in both sexes. For males, we studied the sperm's quantity and quality: although fertile, the *t*-heterozygous male samples had lower motile sperm concentration than wild-type. The t-homozygous male samples also had lower overall sperm concentrations, with these few cells being mostly static and thus unable to achieve fertilisation. For females, we studied offspring production in the lab: surprisingly, t-homozygous females had litter sizes reduced by half compared to wild-type and t-heterozygous mothers. The severe fertility costs observed in both sexes suggest that the t-haplotype may be a promising tool for pest control. At the same time, we would also expect mice to evolve behavioural strategies such as female polyandry or mate choice to avoid t-related fertility costs. We will test this hypothesis in future work.



## Thinking outside the box! Improving the use of bait stations for the management of commensal rodents

Quinn, Niamh<sup>1\*</sup>; Miles, Bosarge<sup>1,2</sup>; Stapp, Paul<sup>2</sup>

<sup>1</sup> University of California, Agriculture and Natural Resources, Irvine, USA

<sup>2</sup> California State University, Fullerton, USA

\* nmquinn@ucanr.edu

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Commensal rodents are one of the most widespread mammals in the urban environment. They pose threats to the public's health and cause structural damage to buildings. Yet, their biology remains understudied. This leads to a lack of understanding of these species, and to incomplete management. Research has shown that commensal rodents enter bait stations less often than expected. This may be one of the reasons why certain populations of commensal rodents never achieve a satisfactory population decrease. Research has also shown that if rodents do not enter bait stations within two weeks of the start of the management program, the program is likely to fail.

We will discuss evaluations of whether modifications to bait stations and luring approaches influence station discovery, entry, bait consumption, or nightly activity of rats. We will also discuss how yard- and landscape-scale characteristics influence visitations to stations by rats in the presence of pets and livestock, nearby food resources, and past and ongoing rodent management.



## To root or not to root? Proximate factors that contribute to wild boar selection of rooting sites in agricultural landscapes

Potočnik, Hubert<sup>1\*</sup>; Jelenko Turinek, Ida<sup>2</sup>; Jaklič, Ana<sup>1</sup>; Črtalič, Jaka<sup>1</sup>; Mlinarič, Eva<sup>3</sup>; Jerina, Klemen<sup>1</sup>; Al Sayegh Petkovšek, Samar<sup>4</sup>; Platovšek, Zarja<sup>4</sup>; Črnec, Katja<sup>4</sup>; Stergar, Matija<sup>5</sup>; Kos, Ivan<sup>1</sup>; Šprem, Nikica<sup>6</sup>; Zgorelec, Željka<sup>6</sup>; Perčin, Aleksandra<sup>6</sup>; Galić, Marija<sup>6</sup>; Rubinić, Vedran<sup>6</sup>; Jurković Balog, Nikolina<sup>6</sup>; Pokorny, Boštjan<sup>4,7</sup>

- <sup>1</sup> University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
- <sup>2</sup> Municipality of Ljubno, Ljubno ob Savinji, Slovenia
- <sup>3</sup> Institute of the Republic of Slovenia for Nature Conservation, Ljubljana, Slovenia
- <sup>4</sup> Faculty of Environmental Protection, Velenje, Slovenia
- <sup>5</sup> Slovenia Forest Service, Ljubljana, Slovenia
- <sup>6</sup> University of Zagreb, Faculty of Agriculture, Zagreb, Croatia
- <sup>7</sup> Slovenian Forestry Institute, Ljubljana, Slovenia
- \* hubert.potocnik@bf.uni-lj.si

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One species that often comes into conflict with humans is wild boar (*Sus scrofa*). In the last decades, various anthropogenic changes in the environment have improved habitat conditions for wild boar, causing an increase in the abundance as well as damage caused by the species in most of its range (including Slovenia). In order to design effective strategies and measures for controlling agricultural damage caused by wild boar, we need to identify and better understand the influential factors, affecting the incidence and degree of damage. One of behavioural characteristics of the species is rooting, which causes the most damage in agricultural areas, and with increasing populations and proximity to humans, wild boar is expected to come into conflict with humans more and more frequently.

We analysed the relationship between damage caused by wild boar rooting and vegetation, soil characteristics, topography, and human disturbance factors. Damage data were collected over two periods: 2000-2008 and 2020-2022. We examined impact of set of environmental/population variables, including perceptual variables in the form of the proportion of urban, agricultural, grassland, forest and other land cover in 1 km and 3 km radius around damage locations. We used generalized regression model, Maxent and generalized linear mixed effect models (GLMMs) to estimate the influence of particular variables in prediction of rooting probability. Furthermore, we examined levels of organic C, total N and some other parameters in soils on rooted locations as well as presence of dominant plant species and their underground parts (root types, stolones, bulbs, and tubers).

Examined models showed that 2-3 environmental/population variables per model had the most important role on the occurrence of wild boar damages in the agricultural land: (i) density of wild boar harvest, density of feeding places (proxies for wild boar population density), (ii) the length of forest edge and density of forest cover (main habitat appearance), and (iii) human footprint index (presence of human disturbance). Damages on grassland occurred in parts of meadows where levels of organic carbon and total nitrogen were significantly higher in comparison with parts of meadows where rooting was not present.



### Mammalian damage estimates in the UK

Smith, Graham<sup>1\*</sup>; Cowan, Dave<sup>2</sup>; Palphramand, Kate<sup>1</sup>

<sup>1</sup> National Wildlife Management Centre, APHA, Sand Hutton, York, UK

<sup>2</sup> Wildlife Consulting, York, UK

\* graham.smith@apha.gov.uk

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Wildlife can cause conflicts with humans through economic losses, impacts on health, the environment and social impacts. Here we have collated, or estimated, the damage for each mammalian species in Britain in each of these categories. A five-point scale of impact was used to categorise each impact, with this starting at no, minimal or less than £10,000 per year, up to widespread severe effects or more than £10 million economic loss per year. The results will be presented in terms of individual species, taxa (e.g. deer or bats) and whether they are native or not. While deer, rats and rabbits are often assumed to be the major pest species, we will put this into perspective with some probable surprises for the impact of other mammal species. Results from a total of 28 terrestrial and 18 bat species that are considered native, 12 introduced species and a few feral species will be presented. A similar exercise was completed in 2013 and we will compare changes over the last 10 years.



### Mitigating the human-wildlife conflict on roads: Czech perspective

Bíl, Michal<sup>1\*</sup>; Kubeček, Jan<sup>1</sup>; Andrášik, Richard<sup>1</sup>; Sedoník, Jiří<sup>1,2</sup>

<sup>1</sup> CDV – Transport Research Centre, Department of Geoinformatics, Brno, Czech Republic

<sup>2</sup> Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Prague, Czech Republic

\* michal.bil@cdv.cz

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Czechia has a dense transportation network. The vast majority of roads are unfenced, which even applies to certain sections of motorways. Along with high populations of wildlife, particularly roe deer and wild boar, this creates a potential for conflicts on transportation infrastructure. Between 2010 and 2019, the number of recorded events in the form of traffic accidents –collisions with wildlife– tripled. Currently, wildlife-vehicle collisions (WVCs) make up 50% of all traffic accidents occurring outside built-up areas. This places a significant administrative burden on traffic police, and as a result, reporting of these events is gradually being discontinued in various districts in the official traffic accident database. Thus, the official data after 2019 shows a decline. On the other hand, data from hunters is unreliable, as hunters are not motivated to report roadkill. Therefore, at CDV, we created a model that aims to estimate the true number of these incidents. From our estimates, it appears that the official WVC numbers reported by the police today represent about half of the actual situation.

To gain an overview of WVCs that are not captured through official reporting channels, we developed a tool to integrate reports of such incidents as well as findings of carcasses. Srazenazver.cz is a platform that integrates official data from the police, traffic information systems, hunters, and also volunteers. As of October 2024, this database contained nearly 160,000 records, with 76% of incidents involving roe deer and 9% wild boar. For road managers, we offer the identification of high-risk locations, which are then remediated. This also applies to WVC. For the national road authority, we developed the kdebourame.cz, which includes WVC hotspots. To detect hotspots, we used our own KDE+ method (https://www.kdeplus.cz/en/).

WVCs are a complex issue, and their solution goes beyond transportation infrastructure, extending into the landscape. It is connected to management practices in hunting, forestry, and agriculture. To offer proven solutions, we test the effectiveness of various mitigation measures. The most widely used, dominant measure in Czechia is odour repellents. We have been studying their effectiveness since 2014. Recent research suggests they may reduce collisions, but only for a limited time frame of several weeks. Since our research was based on carcass collection, we cannot provide insights into animal behaviour or the circumstances of collisions. Therefore, we are preparing testing aimed at understanding animal behaviour near these measures. For this, we are testing tools for analysing GPS collar data and methods for image analysis. However, a vehicle is also required for wildlife collisions. Therefore, we also focus on drivers and methods to influence their behaviour. In our current research, we are testing, using surveys, radars, driving simulators, and other tools, how drivers perceive the "wildlife" traffic sign. The goal of our activities is to describe the wildlife collision issue in Czechia and contribute to reducing the number of these incidents.



### Urban wild boar (*Sus scrofa*) and their management in Zagreb, Croatia

Konjević, Dean<sup>1\*</sup>; Ranogajec, Vlasta<sup>2</sup>; Krapinec, Krešimir<sup>3</sup>; Lugarić, Snježana<sup>2</sup>; Bujanić, Miljenko<sup>1</sup>; Madi, Ivan<sup>4</sup>; Kruc, Miljenko<sup>5</sup>; Reindl, Branimir<sup>6</sup>

<sup>1</sup> University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia

- <sup>2</sup> City of Zagreb, City Office for Economy, Environmental Sustainability and Strategic Planning, Sector for Agriculture, Forestry and Hunting, Zagreb, Croatia
- <sup>3</sup> University of Zagreb, Faculty of Forestry and Wood Technology, Zagreb, Croatia
- <sup>4</sup> Zagreb Zoo, Zagreb, Croatia
- <sup>5</sup> Zagreb Hunting Association, Sesvete, Croatia
- <sup>6</sup> Wilderness Research and Management j.d.o.o., Novoselec, Croatia
- \* dean.konjevic@vef.unizg.hr

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Wild boar (Sus scrofa) is an opportunistic species which thrives in the urban environment, but causes fear, crop damages, wildlife-vehicle collisions, and represents a potential source of zoonoses. In the last decades, the occurrence of wildlife in urban areas is an increasing phenomenon, which leads to the mentioned human-wildlife conflicts. At the same time, one part of the citizens dislikes the presence of wildlife in the city, while the other one supports them with additional feeding. This conflict of opinions makes it harder to implement measures to control their presence. Urban wildlife in the city of Zagreb is partially managed under the Program of game protection. Within that, monitoring of presence and corridors of wild boar and crows, and their control was launched by the City administration in cooperation with Zagreb ZOO, Zagreb Hunting Association, Faculty of Forestry and Wood Technology, and Faculty of Veterinary Medicine, University of Zagreb. In the preparatory phase, locations with reported human-wild boar conflicts were analysed on the field, and photo-traps were deployed. Mobile modular traps with the ability to monitor wild boar via mobile phone were installed on selected locations. Trapped wild boar were chemically immobilized and euthanized, since translocation of alive wild boar is currently prohibited due to the risk of African swine fever transmission. Samples of faeces, blood, nasal and rectal swabs were collected for the purpose of partial health monitoring. Authorized company removed the carcasses. In the same time, hunting associations performed shooting of wild boar in the areas where it was possible according to the legislation. Presence of wild boar in Zagreb during the winter was monitored using the thermal cameras mounted on a drone. Along with cage trapping and shooting, other measures including donation of repellents to citizens, conduction of education, and cleaning of abandoned areas under succession of the secondary growth are also implemented. It is further planned to immobilize five wild boar and mark them with GPS collars to identify corridors used by these animals and connection with nearby Nature Park Medvednica. This integrative approach resulted in removal of 98 wild boar in the period of 1.5 years.



### European experiences with effectiveness of methods for controlling wild boar movement: how to reduce African swine fever spread and agricultural damage?

Platovšek, Zarja<sup>1\*</sup>; Pokorny, Boštjan<sup>1,2</sup>; Al Sayegh Petkovšek, Samar<sup>1</sup>; Broz, Ludek<sup>3</sup>; Bužan, Elena<sup>4,1</sup>; Duniš, Luka<sup>4</sup>; Occhibove, Flavia<sup>5</sup>; O'Mahony, Kieran<sup>3</sup>; Šprem, Nikica<sup>6</sup>; von Essen, Erica<sup>7</sup>; Seferović, Anesa<sup>6</sup>; Zanet, Stefania<sup>5</sup>; Ferroglio, Ezio<sup>5</sup>

- <sup>1</sup> Faculty of Environmental Protection, Velenje, Slovenia
- <sup>2</sup> Slovenian Forestry Institute, Ljubljana, Slovenia
- <sup>3</sup> Institute of Ethnology, Czech Academy of Sciences, Prague, Czech Republic
- <sup>4</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia
- <sup>5</sup> University of Turin, Dipartimento di Scienze Veterinarie, Turin, Italy
- <sup>6</sup> University of Zagreb, Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Zagreb, Croatia
- <sup>7</sup> Stockholm University, Stockholm Resilience Centre, Stockholm, Sweden
- \* zarja.platovsek@fvo.si

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In Europe, wild boar serves as the primary reservoir of African swine fever (ASF), which requires strategies to control disease transmission, including the separation of their populations. We evaluated the effectiveness of different methods for controlling wild boar movement through unpublished field experiences using an *ad hoc* questionnaire distributed to relevant professionals (e.g. veterinary authorities/veterinarians, wildlife managers, wildlife scientists) across Europe. Based on 69 relevant responses from 17 European countries, we gathered evidence on the effectiveness of fences (solid/mesh and electric), natural barriers, and other methods such as repellents/deterrents in affecting wild boar movement, primarily aimed at crop protection and ASF transmission risk reduction.

Questionnaire results showed that solid fences are a very effective tool for crop protection and forest protection (reasonably to completely effective: 85.7% and 90.0%, respectively), and less so for increasing road/railway safety or reducing wildlife-livestock interactions. However, regarding ASF control, solid fences were considered to be very or completely effective for virus control only in 35.7% of cases. Similarly, electric fences are very effective for crop and forest protection (reasonably to completely effective: 91% and 88%, respectively), and to a lesser extent for reducing wildlife-livestock interactions, ASF transmission, or increasing road/railway safety. These findings suggest that while certain types of barriers can reduce wild boar movements, their effectiveness is influenced by numerous factors such as fence characteristics and landscape features. Responses received from different European countries confirmed that, although fences are not fully impermeable, both solid and electric fences can be effective, mainly for crop protection, and to a lesser extent, for ASF control. However, field experiences indicate that successful ASF control not exclusively depends on a specific type of fence, but on the coordinated efforts of different stakeholders, including animal health authorities, local authorities, hunting associations, wildlife managers, farmers, landowners, and the general public.



## Effects of Poland's state border militarization on wildlife in Białowieża Forest

Nowak, Katarzyna<sup>1,2\*</sup>; Bubnicki, Jakub<sup>1,3</sup>; Jaroszewicz, Bogdan<sup>2</sup>; Kołodziej-Sobocińska, Marta<sup>1</sup>; Komar, Ewa<sup>1</sup>; Kuberski, Łukasz<sup>4</sup>; Michielsen, Rosanne<sup>1</sup>; Morawski, Kamil<sup>1</sup>; Nowak, Paweł<sup>2</sup>; Richards, Shane A.<sup>5</sup>; Ruczyński, Ireneusz<sup>1</sup>; Samojlik, Tomasz<sup>1</sup>; Selva, Nuria<sup>6,7</sup>; Śmielak, Michał<sup>8</sup>; Stachowicz, Izabela<sup>9,3</sup>; Zegarek, Marcin<sup>1</sup>; Żmihorski, Michał<sup>1</sup>

- <sup>1</sup> Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland
- <sup>2</sup> University of Warsaw, Faculty of Biology, Białowieża Geobotanical Station, Białowieża, Poland
- <sup>3</sup> Open Science Conservation Fund, Białowieża, Poland
- <sup>4</sup> Forest Research Institute, Department of Natural Forests, Białowieża, Poland
- <sup>5</sup> University of Tasmania, School of Natural Sciences, Hobart, Tasmania
- <sup>6</sup> Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland
- <sup>7</sup> Estación Biológica de Doñana CSIC, Sevilla, Spain
- <sup>8</sup> University of New England, School of Environmental and Rural Science, New South Wales, Armidale, Australia
- <sup>9</sup> University of Łodź, Faculty of Biology and Environmental Protection, Department of Geobotany and Plant Ecology, Łodź, Poland
- \* knowak02@gmail.com

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Linear infrastructure and militarization are ubiquitous features of the Anthropocene. Transboundary areas are increasingly vulnerable to both on account of border security measures implemented by nonenvironment sectors and require special attention for effective biodiversity and connectivity conservation. Białowieża Forest is a transboundary World Heritage site recently split by a state border barrier. Drawing on a variety of methods including transects, camera traps, snow-tracking, and audio recording, we are attempting to evaluate how militarization of the Polish-Belarussian border is affecting wildlife and the forest. Transect data from 10 locations along the border and 3 from "control" roads suggests that wildlife avoids both types of structures but avoidance of the border is stronger. At the border, there is relatively more human activity (as determined by human sign encounter rates and camera trap rates) and less animal sign and activity, while near roads, animal sign is concentrated at intermediate distances. Human signs and sounds are most pervasive along the border but fall off with increasing distance from the edge. Observations from snow track surveys suggest that some opportunistic mesocarnivore species are relatively undeterred by the barriers and attracted to military outposts for feeding opportunities. Signs (tracks) of domestic animals, especially cats, also appear near outposts indicating increased domestic animal-wildlife-human interface. A mix of field methods under challenging conditions is yielding insights which can help inform further monitoring and mitigation.



### Comparative analysis of expert and citizen science reveals differences in occurrence patterns of lynx and wolf in Lower Saxony, Germany

Ludolphy, Catharina<sup>1\*</sup>; Gräber, Reinhild<sup>2</sup>; Strauß, Egbert<sup>2</sup>; Klages, Inga<sup>2</sup>; Reding, Raoul<sup>3</sup>; Anders, Ole<sup>4</sup>; Middelhoff, Tomma Lilli<sup>4</sup>; Balkenhol, Niko<sup>1</sup>

<sup>1</sup> University of Goettingen, Wildlife Sciences, Goettingen, Germany

- <sup>2</sup> University of Veterinary Medicine Hannover (Foundation), Institute for Terrestrial and Aquatic Wildlife Research, Hannover, Germany
- <sup>3</sup> Hunting Association of Lower Saxony, Hannover, Germany
- <sup>4</sup> Harz National Park, St. Andreasberg, Germany
- \* catharina.ludolphy@uni-goettingen.de

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Protective legislation, improved public opinions, and measures fostering the coexistence of large carnivores in our anthropogenic landscapes have aided the re-establishment of wolves and lynx in Germany. As their return is accompanied by conflicts, much effort is put into observing the recolonization process throughout the country. Our study focuses on contrasting two different data-gathering approaches for monitoring carnivore presence in Lower Saxony, Germany: (i) the official monitoring approach, which combines public reports that are validated by experts with systematic methods, such as scat searches; (ii) an annual wildlife survey in the form of a questionnaire sent to hunting district owners and leaseholders, requesting information on several hunting and wildlife-related topics, including the presence of both large carnivore species in their districts.

Though both approaches have their strengths and weaknesses, a preliminary evaluation of wolf data published in 2017 showed high spatial congruence between the datasets gathered from these two methods. This suggests that their combined use could potentially yield more accurate information on species occurrences. However, this preliminary evaluation focused only on wolves and was only done for the first two years in which questions related to carnivore presence were incorporated within the wildlife survey. We therefore compared the information of both approaches on the occurrences of lynx and wolf in terms of their general agreement, their spatial patterns, and also their temporal dynamics using an internal classification scheme. Similar information was provided by both approaches regarding the general development of lynx and wolf occurrences across Lower Saxony. However, spatial differences were mainly found at the periphery of their confirmed distribution ranges. Regarding temporal dynamics, the wildlife survey provides information about two years ahead of the official monitoring programs. The species-related differences in the accuracy of our classification system may be attributed to different attitudes towards the two species. Ultimately, our findings suggest that both monitoring approaches are complementary and the assessment of species occurrences should involve insights from both datasets.



### Coexistence of wolves and herders within Mongolia's ancient pastoral traditions

Dolphin, Jeff<sup>1\*</sup>; Mazzamuto, Maria Vittoria<sup>1,2</sup>; Gansukh, Sukhchuluun<sup>3</sup>; Koprowski, John L.<sup>1,4</sup>

<sup>1</sup> University of Wyoming, Haub School of Environment and Natural Resources, Laramie, WY, USA

<sup>2</sup> University of Turin, Department of Life Sciences and Systems Biology, Turin, Italy

<sup>3</sup> Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia

<sup>4</sup> University of Arizona, School of Environment and Natural Resources, Tucson, AZ, USA

\* jdolphin24@icloud.com

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Mongolia's deep-rooted tradition of pastoralism, tracing back over 5,500 years, reflects a resilient bond with its vast landscapes and wildlife. This connection plays a critical role in the coexistence of livestock and predators, shaping strategies to protect herds from threats across Mongolia's expansive steppe. As of 2024, Mongolia's population of 3.5 million supports approximately 64 million livestock, with herding sustaining over a third of the nation's workforce. Wolves, which account for an estimated 15,000 livestock losses each year, are classified as near-threatened in Mongolia due to overharvest, often related to international trade. However, they present a major challenge for rural communities. Distinct from other regions, Mongolia has no formal compensation programs for depredation losses, pressing herders to rely on self-devised methods of prevention.

From 2022 to 2024, we conducted surveys with livestock owners in proximity to Bogd Khan Mountain, one of the oldest protected areas in the world, and UNESCO Biosphere Reserve. We evaluated the impacts of wolves on herders and the adaptive strategies they employ. Additionally, we conducted a camera trap survey within the protected area to examine the abundance and activity of wolves in relation to livestock (horses and cattle) and natural prey (wapiti and Siberian roe deer).

Our findings indicated that wolf abundance was not related to the number of livestock present; in fact, there was low temporal overlap with cows and moderate overlap with horses. Instead, the abundance of wolves was positively associated with the availability of natural prey. Moreover, the activity of wolves showed higher overlap with that of natural prey compared to livestock, displaying similar patterns with two activity peaks occurring at dawn and dusk. The results of the herders' survey revealed a variety of approaches to managing wolves and herds. These included rotational grazing, the use of guard dogs, and culturally significant methods such as traditional wolf hunting. Additionally, the sociodemographic characteristics of the interviewees influenced their perceptions of wolves and their herd management strategies. This study reveals the complexities of balancing traditional practices with emerging ecological pressures, demonstrating how Mongolia's pastoral communities navigate predator coexistence amid modern economic and environmental challenges. These findings offer valuable insights into community-driven conservation practices in pastoral landscapes of countries with an emerging economy.



### Human-leopard conflict in Ahmednagar, Pune, and Nashik Districts of Maharashtra, India: patterns and community perceptions on conservation

Kurhe, Anil<sup>1\*</sup>; Raut, Ranjit<sup>2</sup>; Nirbhawane, Ashutosh<sup>1</sup>

<sup>1</sup> Savitribai Phule Pune University, Pune-Padmashri Vikhe Patil College of Arts, Science and Commerce Pravaranagar, Department of Zoology, Tal. Rahata, Dist. Ahmednagar, MS, India

<sup>2</sup> Yogeshwari Mahavidyalaya, Department of Zoology, Ambajogai, Dist. Beed, MS, India

\* anil.kurhe@gmail.com

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Human-animal conflict represents a significant challenge in regions where human populations expand into wildlife habitats. This leads to increased encounters with species that can threaten livelihoods, property, and safety. Human-leopard conflict has become a growing concern in the regions of Ahmednagar, Pune, and Nashik in Maharashtra. Indian leopard (*Panthera pardus fusca*) attacks on humans and livestock have a long history and are increasing in severity due to habitat encroachment, resource competition, and the leopards' adaptable nature, which brings them into close proximity with human settlements. The purpose of this study was to explore conflict patterns and to examine local community perceptions of conservation strategies aimed at mitigating these interactions.

The research identifies prevalent conflict forms, including livestock predation, occasional human attacks, and property damage, which have resulted in economic losses, fear, and retaliatory actions. Conflict patterns reveal that leopards, driven by habitat fragmentation and prey shortages, increasingly venture into peri-urban and rural areas, making domestic animals easy targets. The study also investigates demographic, cultural, and economic factors that shape community attitudes towards leopards and assesses the effectiveness of current conservation measures. Community perspectives reflect a range of responses, from antagonism to conditional support for leopard conservation, influenced by the perceived effectiveness of mitigation measures and compensation schemes. Many local people express frustration with current policies, citing delayed compensation and insufficient support in the wake of leopard encounters. However, where educational outreach and rapid response programs are in place, there is greater tolerance and support for conservation efforts. Study highlights the need for an integrated conservation framework that involves local communities in decision-making, enhances compensation processes, and promotes awareness campaigns on safe coexistence. Understanding local perceptions is essential to formulating strategies that balance human livelihoods with leopard conservation, creating a sustainable model for wildlife management in Maharashtra's multi-use landscapes.



### Towards understanding and resolving conflicts of bat presence and maintenance of buildings of cultural heritage in Slovenia

Presetnik, Primož<sup>1\*</sup>; Zamolo, Aja<sup>1</sup>; Erbida, Nina<sup>1</sup>; Trošt, Tadeja<sup>1</sup>

<sup>1</sup> Centre for Cartography of Fauna and Flora, Ljubljana office, Ljubljana-Šentvid, Slovenia

\* primoz.presetnik@ckff.si

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Castles, churches and other buildings of cultural heritage (BCH) play an essential role for a number of bat species across Europe. Maintaining bat roosts in such manmade structures is a very specific conservation problem, as owners or managers of BCH need to be persuaded to accept bats in their buildings, mostly in connection with the additional workload caused by cleaning bat guano. Addressing this problem is one of the focuses of the *LIFE integrated project for enhanced management of Natura 2000 in Slovenia* (LIFE-IP NATURA.SI). As a strong scientific basis is an integral part of nature conservation communication, we have analysed data on the presence of bats in BCH in Slovenia (an area of 20,271 km<sup>2</sup> or about 250×150 km).

Over the last two decades, more than 1,700 BCHs were surveyed. Bats or evidence of their presence were found in 80% of them, i.e. in 1,350 churches and 44 castles. Of the 32 bats species present in Slovenia, 23 were recorded and, to our knowledge, BCH are crucial roosts for 10 bat species (*Rhinolophus hipposideros, R. ferrumequinum, R. euryale, Myotis myotis, M. blythii, M. emarginatus, Plecotus macrobullaris, P. austriacus, Miniopterus schreibersii*). All these bat species and their habitats are protected and classified as endangered or vulnerable in the Slovenian Red List. Maternity roosts were confirmed in 631 BCH (37%), and as more than one bat species may use the same building, 780 nursery groups were recorded. Groups of bats usually consisted of less than 100 adults; however, exceptionally (in 14 buildings) there were more than 500 or even more than 2,000 adults present. The second part of the project was a survey of over 400 bat roosts in the BCH, where we recorded details of the microlocation and amount of bat guano in the BCH and interviewed their managers on the subject. The third part of the project involved small-scale improvements to selected bat roosts to test the effectiveness of some technical measures regarding limiting bat guano loads. Finally, we will discuss long-term options to help BCH managers maintain peaceful coexistence with bats.



### **ProVeBirD** – **Protection of vegetables from bird damages**

Göbel, Florian<sup>1\*</sup>; Esther, Alexandra<sup>1</sup>

<sup>1</sup> Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Braunschweig, Germany \* florian.goebel@julius-kuehn.de

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Bird damage remains an important and unresolved issue in plant protection. While scientific evidence on the extent of damage caused by bird feeding and droppings is limited, numerous reports from vegetable producers highlight the severity of the problem, raising concerns about the economic viability of certain crops. Additionally, wild birds pose health risks by transmitting pathogens such as *Salmonella* through faeces to humans and animals, creating potential dangers for both producers and consumers beyond mere aesthetic concerns. Despite its relevance to vegetable farming, there is a lack of comprehensive insights into effective and economically viable protection measures against bird damage. The ProVeBirD project aims to identify potential losses in major and specialty crops while developing a practical guide for organic vegetable farming systems.

We conducted a survey of German farmers which revealed that bird feeding affected all types of vegetables, particularly leaf, root, tuber, and fruit vegetables. Crows (Corvidae) were identified as the most damaging bird family, followed by pigeons (Columbidae) and geese (Anatidae). Average damages per crop and farm site were estimated at approximately 20%. Nearly half of the participants reported their losses as significant or very significant, with almost two-thirds noting a negative trend, i.e. increasing damages over recent years. Most farmers were unaware of the effectiveness of countermeasures; however, many reported successes with hunting, protective nets, and acoustic methods such as propane cannons or distress calls, while traditional scarecrows were largely considered ineffective. These results build the basis for the next ongoing project phase in which we conduct field trials to test the effectiveness of several protective measures for vegetables against bird damage.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and other Forms of Sustainable Agriculture.



### Habituation of pigeons to hawk's presence during the control programs in urban areas

Buet, Anja<sup>1</sup>; Reindl, Branimir<sup>2\*</sup>; Bujanić, Miljenko<sup>1</sup>; Konjević, Dean<sup>1</sup>

<sup>1</sup> University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia

<sup>2</sup> Wilderness Research and Management j.d.o.o., Novoselec, Croatia

\* branimir.re@gmail.com

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The increasing populations of pigeons (*Columba livia*) in urban areas represents a growing problem from the perspective of public health and potential contamination and damages of buildings, monuments, etc. The aim of the study was to analyse and observe the effect of using trained raptors on the population of urban pigeons at the factory Valipile in Dumovec, Zagrebačka county, Croatia. One of the main concerns was possible contamination of poultry feed by pigeon droppings and consequent transmission of pathogens into the production. The study was divided in three phases, during September and October 2024. Phase one included monitoring of undisturbed pigeons and crows in the area, for eight days. In the phase two, raptors (i.e., Harris's hawks [*Parabuteo unicinctus*]) were released four times (2x2) with varying intervals. There was a 5-day interval between two raptors flights. The third phase started five days after the second one and included four ousterings. Intervals between first and second, and third and fourth one was one day, while the interval between second and third one was seven days. The presence of pigeons was monitored non-invasively using three photo-traps.

In the first phase, we observed high numbers of pigeons from the morning until midday (8 a.m. till 1 p.m.). Pigeons were more present at the factory in October, probably due to the arrival of the harvested corn and pigeon preparations for winter. During the second phase of the study, after each hawk' flight a sharp decline in pigeon numbers was observed but the following day pigeons returned in "normal" numbers. In the first part of the third phase, hawks have induced decline in pigeon numbers during the flight day, while in the second part this decline was observed only a day after. During the third phase of the research, it was observed that during the hawks' presence pigeons used to take a so-called safety zone, keeping at a safe distance from the hawk, but remaining at the study site. The results of the study point to the habituation of pigeons to the presence of raptors. It was confirmed that falconry alone is not efficient enough for a longer period and should be complemented with other methods of bird control.



### Data papers as a valuable reporting tool to improve the management and conservation of vertebrate populations in Europe

Illanas, Sonia<sup>1</sup>; Fernández-López, Javier<sup>1,2</sup>; Vicente Baños, Joaquin<sup>1</sup>; Ruiz-Rodríguez, Carmen<sup>1</sup>; López-Padilla, Sergio<sup>1</sup>; Sebastián-Pardo, Mario<sup>1</sup>; Preite, Ludovica<sup>1,3</sup>; Gómez-Molina, Azahara<sup>1</sup>; Acevedo, Pelayo<sup>1</sup>; Blanco-Aguiar, José Antonio<sup>1\*</sup>

<sup>1</sup> Instituto de Investigación en Recursos Cinegéticos (IREC), Ciudad Real, Spain

<sup>2</sup> Universidad Complutense de Madrid (UCM), Madrid, Spain

<sup>3</sup> European Food Safety Authority (EFSA), Parma, Italy

\* joseantonio.blanco@uclm.es

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Biodiversity data are essential for wildlife management and conservation. The open access to standardized information on wildlife distribution and abundance, and more generally, biodiversity, favour the use of large databases to generate new research at large scales (but also locally) that otherwise would not be possible. In addition to improving the quality of available data, data papers are key to adequately document data collection protocols and subsequent processing, facilitating the recognition of authors and data owners while dealing with limitations on data privacy.

We review the publication trends of data papers on vertebrates in Europe; as a case study, we present the reporting of hunting statistics of big game species in Spain, describing the procedures used to generate more than 1 million records. The datasets provide long-term information (2013-2022) of the only-presence of eight wild ungulates and red fox derived from harvest data in a grid of 5x5 km for mainland Spain (21,836 cells). The collected data are presented on a yearly basis as well as grouped into two monitoring periods. The spatial resolution (as well as the selection of the specific grid used) and periods match the characteristics of obligatory data reporting on biodiversity by countries to the EU, as required by the Habitats Directive. This case illustrates the utility of data papers to publicly provide information that subsequently can easily contribute to data reporting by countries, facilitating the early mobilization of data and reducing workload by national administrations (which often struggle to transfer complete and up to date quality data). We conclude that the publication of data papers by the scientific community following EU standards for biodiversity data reporting are set to become essential in the frame of future schemes of biodiversity monitoring in Europe, such as the proposed EU Biodiversity Observation Coordination Centre (EBOCC). Finally, some proposals to encourage collaborative science and the reuse of biodiversity data are discussed.



## Optimizing wildlife monitoring through cost-effective distribution of observatories

Fernández-López, Javier<sup>1\*</sup>; Blanco-Aguiar, José Antonio<sup>1</sup>; Vicente Baños, Joaquín<sup>1</sup>; Acevedo, Pelayo<sup>1</sup>

<sup>1</sup> Instituto de Investigación en Recursos Cinegéticos (IREC), Ciudad Real, Spain

\* Javier.FLopez@uclm.es

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Effective wildlife management and conservation require reliable information on species distribution and abundance. Here, we present a scalable and transferable methodology designed to capture the ecological and management heterogeneity of any study area, thereby optimizing wildlife monitoring programs. By delineating homogeneous sampling units based on key environmental and management factors, this approach enables focused monitoring in a strategically selected subset of areas ("observatories"), ensuring robust data collection while optimizing resource use. Our procedure consists of three stages. First, we apply multivariate techniques to assess the main environmental, management, or biodiversity patterns across the study area. Second, clustering methods are used to identify homogeneous regions or management units. Finally, optimization algorithms establish a network of well-distributed monitoring points to ensure a cost-effective solution. This structured, adaptable framework is highly applicable across regions and scales.

We tested this methodology in two real-world case studies: (i) a management network for game ungulates in Castilla-La Mancha, central Spain, and (ii) a national-scale monitoring program for wild mammals across Spain. In both cases, we first identified and categorized homogeneous ecological regions using climate and vegetation data. We then incorporated management characteristics within each region or biodiversity indices, such as species richness and rarity, to form a network of representative observatories. This approach enables targeted data collection from a limited number of strategically distributed sites, capturing population trends and biodiversity metrics across broader areas. Our stratified, observatory-based framework provides a flexible and cost-effective solution adaptable to multiple species and regions globally. This protocol is well-suited to guiding wildlife management decisions at local, regional, and national levels, maximizing efficiency and representativity across diverse ecological contexts.



## Rewilding herbivores: could fertility control and other interventions help?

Massei, Giovanna<sup>1\*</sup>; Gordon, Iain J.<sup>2-5</sup>; Wimpenny, Claire<sup>6</sup>; Manning, Adrian D.<sup>2</sup>

<sup>2</sup> The Australian National University, Fenner School of Environment and Society, Acton, Australia

<sup>3</sup> The James Hutton Institute, Dundee, UK

<sup>4</sup> Central Queensland University, Townsville, Australia

<sup>5</sup> James Cook University, College of Science and Engineering, Townsville, Australia

<sup>6</sup> Office of Nature Conservation, ACT Government, Dickson, Australia

\* GMassei@botstiber.org

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Rewilding is becoming a mainstream approach to ecosystem restoration. Most rewilding projects include populations of large free-roaming herbivores that can grow fast. As this growth can undermine long-term rewilding goals, there is a pressing need to consider if, when, and how to intervene in the management of large herbivore populations during the rewilding process. In parallel, there is growing demand for non-lethal population management options. We will examine how "no intervention" as well as four types of nature-mimicking interventions could be applied to rewilding processes. 'No intervention' influences some of the natural processes that govern herbivore population size and distribution. Where top-down processes are absent, bottom-up processes will eventually predominate and often result in starvation and ultimately death. This impacts animal welfare, public trust and risks ecosystem degradation. Culling mimics the effects of emigration, predation, and disease and affects animal behaviour. Interventions that mimic the presence of predators can be used to create landscapes of fear: anti-predator behaviour can affect ecosystem structure, composition, function, and restoration. Translocation can have the same effect of culling and fertility control but also increased risk of disease transmission, and significant welfare costs. Fertility control is achieved via surgical sterilisation, hormonal implants, and injectable contraceptive vaccines. Fertility control mimics the outcome from predation, disease, and density dependent effects that lower birth rate and can reduce costs, negative welfare issues, and increase public support for rewilding. No single intervention is likely to work alone, and regimes combining different interventions will be needed throughout the rewilding process. We will explore examples of these interventions to reduce environmental, economic, animal welfare, social and cultural impacts, and crucially contribute to maintaining local support for rewilding projects.



<sup>&</sup>lt;sup>1</sup> Botstiber Institute for Wildlife Fertility Control, Department of Environment & Geography, University of York, York, UK

### Monitoring deer browsing and density in Mediterranean forests: impacts of introduced fallow deer are larger than those of native roe deer

Fattorini, Niccolò<sup>1,2\*</sup>; Bonari, Gianmaria<sup>1,2</sup>; Fini, Giovanni<sup>1,2</sup>; Angiolini, Claudia<sup>1,2</sup>; Ferretti, Francesco<sup>1,2</sup>

<sup>1</sup> University of Siena, Department of Life Sciences, Siena, Italy

<sup>2</sup> National Biodiversity Future Center, Palermo, Italy

\* niccolo.fattorini@unisi.it

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Herbivory by wild ungulates may trigger significant impacts on vegetation, and recent studies suggest scale-dependent effects of population density. Yet, there is no agreement on whether impacts should be related to densities and about the scale of effect. Gaining knowledge on such issues would be crucial to target the appropriate spatial scale over which effective ungulate management should be conducted. Additionally, deer browsing pressure in Mediterranean forests has been understudied compared to temperate forests. During 2019-2023, we investigated whether multi-scale, spatiotemporal variations in densities of introduced fallow deer (*Dama dama*) and native European roe deer (*Capreolus*) drove the browsing impact on forest vegetation, within a Mediterranean protected area.

We found no effect of deer densities on browsing at the finest, sampling plot-scale. Higher browsing pressure was associated with greater fallow deer densities at both the home range and study area scales, but not with roe deer densities. We obtained the same results when considering browsing pressure on forest patches as a whole and separately on the dominant woody plants in the study area (*Quercus ilex; Phillyrea* spp.). Browsing indices decreased by 75% in 2019-2023, matching the steady decline in fallow deer population density (–25%). Throughout the study period, fallow deer density in any year was unrelated to relevant culling pressure in the previous year. Conversely, the decreased fallow deer density was related to the increased grey wolf (*Canis lupus*) numbers (for which the fallow deer is a major prey), suggesting consumptive effects by predators achieving top-down control of deer impact on vegetation.

Our study offers practical insights into population monitoring and management of wild ungulates, as well as their impact on natural ecosystems. First, we showed how the population density of the introduced deer species –but not that of the native one– was related to browsing impact on forest vegetation. Hence, browsing pressure by wild ungulates can be used as a surrogate for monitoring spatiotemporal variations in population densities, although we suggest caution in contexts with co-occurring species, as not all of them have the same impact. Moreover, this finding emphasises the major role of introduced ungulates in eliciting impacts on natural habitats, advocating proper management actions. Second, we identified the spatial scales at which deer densities would be more likely to impact forest vegetation. Third, we showed a potential example of recolonizing predators helping in the control of both ungulate densities and, as a consequence, their impacts on ecosystems.



## Finding the optimal population density estimation method for roe deer in open areas

Tóth, Gergely<sup>1\*</sup>; Katona, Krisztián<sup>1</sup>

<sup>1</sup> Hungarian University of Agriculture and Life Sciences, Institute for Wildlife Management and Nature Conservation, Department of Wildlife Biology and Management, Gödöllő, Hungary

\* tothgergely0148@gmail.com

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Monitoring population density of game populations is a basic task for game managers. However, it is not easy to find a relatively cost-effective, simple method which can be used widely among practitioners. European roe deer (*Capreolus capreolus*) populations have been increasing in Europe (also in Hungary) in the last decades. However, game managers rarely perform reliable counts of their populations, which leads to the underestimation of their population size and under-harvesting of the species, causing the wastage of important natural resources. Therefore, our aim was to identify the most suitable, cost-effective technique for roe deer density estimation in Hungary, where the species mainly occupies the lowland, sparsely forested, high-visibility flat areas.

We compared the census data obtained by: (i) the total counting in the daytime strip transect, (ii) the night spotlight strip transect, and (iii) the total counting of the sample areas with thermal camera from observation points within 0-250 m and 0-500 m ranges. The study was carried out in seven hunting areas. Our results supported the underestimation of roe deer populations obtained by classic methods. We revealed that using the thermal camera within 0-250 m and the spotlight method in the same range gave the statistically highest population density values without significant difference between them. The lowest mean value was obtained in case of the daytime strip transect, which results in underestimation of the population density in all cases. The thermal imaging method gave significantly lower values for the larger distance (250-500 m) than for the range of spotlighting (250 m). The night spotlight strip transect method and the counting from observation points with a thermal camera, both to 250 m, provided the highest values, thus they are recommended to be used for determining roe deer population density in open flat areas. Although they require a comparable amount of human resources and time, the spotlight method is well-suited for widespread use by wildlife managers due to the high costs of thermal cameras.



## Management of red deer (*Cervus elaphus*) population in the hunting ground Đerdap (Eastern Serbia)

Gačić, Dragan<sup>1\*</sup>; Mladenović, Slavko<sup>2</sup>; Hadži-Pavlović, Mihajlo<sup>1</sup>

<sup>1</sup> University of Belgrade, Faculty of Forestry, Belgrade, Serbia

<sup>2</sup> Ministry of Environmental Protection, Sector for Supervision and Preventive Action, Belgrade, Serbia

\* dragan.gacic@sfb.bg.ac.rs

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Reintroduction of red deer (*Cervus elpahus*) into the forest complexes of Eastern Serbia began in 1960, while the Derdap hunting ground was established in 1990, and its border coincides with the border of the Derdap National Park established in 1974. This hunting ground comprises a narrow-forested belt of irregular shape along the Danube (total area 63,730 ha, length 100 km, width from 2 to 8 km), located at an altitude of 63 to 803 m. The hunting-productive area for red deer covers 20,000 ha, and its estimated number was 360 individuals in spring 2024 (sex ratio approx. 1:1).

The aim of this study was to analyse the development dynamics of the newly established red deer population in the wider Derdap area (period 1960-2020), focusing on the trophy structure and age of individuals hunted in the Derdap area in two periods: 1993-2002 (54 trophies) and 2007-2020 (73 trophies), respectively. The age was estimated by the skilled commissions based on the tooth wear. The trophies were evaluated according to the International Council for Game and Wildlife Conservation (CIC) method. Each parameter was analysed using descriptive statistics by determining the range of variation, arithmetic mean, standard deviation, and coefficient of variation. A simple regression analysis was used to establish a correlation between the antler parameters and the age of the individual. In addition, relevant information was collected from annual and long-term hunting ground management plans and from records of works carried out in each hunting year. In 2017 and 2018, intensive field research was carried out as a part of the SRBREDDEER project, funded by the Forest Directorate of the Ministry of Agriculture, Forestry and Water Management of Republic of Serbia.

Reintroduction of red deer in the wider Đerdap area was very successful. The first period (1993-2002) was not favourable for planned and rational wildlife management due to the economic crisis caused by international sanctions and armed conflicts during 1990s. The second period was more favourable, especially in the last three analysed years (2018-2020). Red deer losses recorded in the first period were significantly higher, mainly due to poaching and predation by wolves. Between both periods, there was neither statistically significant difference in the mean trophy score (167.1 vs. 167.8 CIC points) nor in the mean age of stags hunted (7.0 vs. 6.9 years). In both periods, there was a large proportion of middle-aged individuals in the harvest (age class 5-8 years), especially in the second period. The trophy structure in these periods was: gold (7.4% vs. 1.4%), silver (11.1% vs. 26.0%), and bronze medal (31.5% vs. 23.3%). The proportion of antlers without a medal was the same in both periods (around 50%). The highest red deer trophy score in the Đerdap hunting ground was 232.1 CIC points (13-year-old individual). The basic recommendations for improving the current state of the red deer population are: (i) the formation of a hunting and breeding area and the implementation of unique objectives and breeding measures by all users of hunting grounds (i.e., both hunting associations and public enterprises); and (ii) enabling quality red deer males to reach at least 11-12 years of age.



## Comparing methods for measuring the distributions and densities of wild boar for disease management

Beatham, Sarah<sup>1\*</sup>; Thomas, Erin<sup>1</sup>; Henderson, Dan<sup>1</sup>; Cunningham, Eloise<sup>1</sup>; Smith, Graham<sup>1</sup>

<sup>1</sup> Animal and Plant Health Agency, Sand Hutton, York, UK

\* sarah.beatham@apha.gov.uk

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Wild boar has negative effects on the environment, economy, and poses health risks to both livestock and humans. In Europe, the increasing spread of African swine fever among populations of wild boar and domestic pigs is currently a significant concern. To effectively model epidemiology and develop appropriate contingency plans, it is essential to gather accurate data regarding the distribution, movement patterns, and population densities of wild mammals. The methods employed for data collection must be practical, timely, and cost-effective. Recent technological advancements have broadened the range of available methods, which need to be evaluated to determine the most suitable option for the species, environment and objectives concerned. Here we present a comparison of results from trials conducted on three distinct wild boar populations in the UK, utilizing techniques such as thermal imaging, distance sampling, thermal drones, the Random Encounter Model with camera traps, and acoustic monitoring. The calculated wild boar densities across the various sites were found to be comparable among the different methods employed; however, each method had unique advantages and disadvantages, which are discussed in relation to their applicability to different species, environments, and study objectives.



### From pilot study to cornerstone conservation and management tool: two decades of genetic monitoring of brown bear in Slovenia

Skrbinšek, Tomaž<sup>1,2\*</sup>; Bartol, Matej<sup>3</sup>; Boljte, Barbara<sup>1</sup>; Hrga, Nuša<sup>1</sup>; Hvala, Tilen<sup>3,4</sup>; Majić Skrbinšek, Aleksandra<sup>1,2</sup>; Pazhenkova, Elena<sup>1,2</sup>; Predalič, Miha<sup>3</sup>; Simčič, Gregor<sup>1</sup>; Konec, Marjeta<sup>1,2</sup>

- <sup>1</sup> DivjaLabs Ltd., Ljubljana, Slovenia
- <sup>2</sup> University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
- <sup>3</sup> Slovenia Forest Service, Ljubljana, Slovenia
- <sup>4</sup> Slovenian Hunting Association, Ljubljana, Slovenia
- \* tomaz.skrbinsek@bf.uni-lj.si

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For many wildlife species, genetic monitoring is the only approach that can provide robust and precise tracking of population abundance, although its practical application can be challenging. In Slovenia, we have developed and implemented genetic monitoring tools over the past two decades to track brown bear population at the national level. Following a pilot study in 2004, a national-level sampling in 2007 established the value of genetic monitoring. Since then, it has become an essential part of brown bear management in Slovenia, with samplings conducted every eight years using the citizen-science approach. The methods were constantly improved to take advantage of the rapid development of molecular genetics, and results are carefully and thoroughly provided to all stakeholders and participants.

In the autumn of 2023, during the third monitoring session, we collected an impressive 2,864 noninvasive genetic samples over three months, surpassing our target of 2,500. The majority of samples were collected by hunters and foresters. We genotyped 2,500 samples, achieving a 70% genotyping success rate. Genotyping using high-throughput sequencing enabled rapid and cost-effective analysis, with laboratory work and genotyping completed within eight months of sampling. The entire study was finalized and reported within ten months of completed sampling.

We documented continued population growth, a further expansion of the species towards the Alps, and a sex ratio similar to previous surveys (61.7% of females). The capture-mark-recapture estimated minimum annual population size at the end of 2023 (after annual mortality but before reproduction) was 739 individuals (ranging from 695 to 797). As the registered mortality in 2023 was 264 individuals, we can add that figure to estimate the maximum annual population size in spring 2023, i.e., at 1001 bears (957 – 1061).

The Slovenian brown bear monitoring program exemplifies a successful transition from a scientific pilot study to a well-established and cost-effective routine monitoring, crucial for the effective management and conservation of the species. The knowledge gained and methodology advancements can be applied to establish similar genetic monitoring programs for other populations and species.



## Golden jackal in Slovenia: from protected species to sustainably managed newcomer predator

Potočnik, Hubert<sup>1\*</sup>; Črtalič, Jaka<sup>1</sup>; Pokorny, Boštjan<sup>2,3</sup>; Flajšman, Katarina<sup>3</sup>; Kos, Ivan<sup>1</sup>

<sup>1</sup> University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia

<sup>2</sup> Faculty of Environmental Protection, Velenje, Slovenia

<sup>3</sup> Slovenian Forestry Institute, Ljubljana, Slovenia

\* hubert.potocnik@bf.uni-lj.si

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During the last decades, golden jackal (*Canis aureus*) population in South-eastern Europe has expanded across the Balkan Peninsula and also into Central, Eastern, and Northern Europe. The species is highly adapted to thrive in human-dominated landscapes where it takes advantage of human presence and a large amount of accessible food sources. These, together with changes in land use, prey/predator abundance and climate, have likely facilitated the expansion process. The spread of the jackal has raised numerous conservation and management issues, while the emerging ecological role of the species in the areas of recent range expansion is barely known. A similar trend has been also observed in Slovenia, which was confirmed by a rapid increase in the jackal distribution range and numbers. In 2004, the golden jackal became protected species in Slovenia. In 2014, however, the species was declared a game species, but at the same time remained on the list of protected species, which caused a legislative collision. Such status had disabled active management of the species. In order to gain basic knowledge about the population and provide the scientific background for appropriate management, a nationwide monitoring was established with the aim of determining the distribution, abundance, population trends, and potential expansion of the golden jackal in Slovenia, resulting in its removal from the list of protected species in 2019.

An important part of the monitoring is based on the integration of hunters –as very important citizen scientists- who are obliged to provide game monitoring as a public service. For this reason, new online monitoring module was developed which enabled instant recording of georeferenced signs of jackal presence of various types including photo-material and records from bioacoustic stimulation method (BAM), and harvest data have been included since 2020 when hunting of golden jackal was allowed for the first time. We developed an ad-hoc expert habitat suitability model for golden jackal in order to determine areas with intensive monitoring (including BAM sessions) versus less suitable areas with passive monitoring only. The first territorial group of jackals in Slovenia was confirmed in 2009, however in 2019 territorial response (howling) of jackals in 374 cells (3x3 km) were registered during the monitoring season. Sharp increase in abundance was also indicated with harvest data: 179 jackals were harvested in 2020, and this figure increased to 1086 harvested individuals in 2024, respectively. The jackal distribution range as a legally neo-native, newcomer predator overlaps with the ranges of two other native canid species, i.e., grey wolf (Canis lupus) and red fox (Vulpes vulpes). We examined the extent of spatial segregation between wolves and jackals and developed habitat suitability models for each species. All data on wolf's and jackal's distributions in Slovenia were collected from the national monitoring databases. For red fox, data on relative abundances were taken from the hunting records and compared to jackal distribution.



## The recovery of pine marten (*Martes martes*) in Britain and implications for grey squirrel (*Sciurus carolinensis*) management

Scopes, Eleanor R.<sup>1\*</sup>; MacPherson, Jenny<sup>2</sup>; Wright, Patrick G.R.<sup>2</sup>; McNicol, Catherine M.<sup>3</sup>; Kingscott-Edmunds, Jamie<sup>4</sup>; Kerecsenyi, Nora<sup>5</sup>; Guy, Matt<sup>6</sup>; Bellamy, Chloe C.<sup>5</sup>; Weaver, Josh<sup>6</sup>; Rae, Nicola<sup>5</sup>; A'Hara, Stuart<sup>5</sup>; Ham, Cally<sup>6</sup>

<sup>1</sup> Forest Research, Forestry Commission National Office, Bristol, UK

<sup>2</sup> Vincent Wildlife Trust, Eastnor, Ledbury, UK

<sup>3</sup> British Association for Shooting and Conservation, Marford Mill, Wrexham, UK

<sup>4</sup> Gloucestershire Wildlife Trust, Gloucester, UK

<sup>5</sup> Forest Research, Northern Research Station, Roslin, UK

<sup>6</sup> Forest Research, Alice Holt Lodge, Farnham, UK

\* ellie.scopes@forestresearch.gov.uk

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In Britain, invasive grey squirrels (*Sciurus carolinensis*) cause extensive damage by bark stripping, reducing the value of trees for forestry and broader ecosystem services. Due to these negative impacts and invasive status, widespread management and control is undertaken by landowners to reduce squirrel abundance, but this requires significant time, effort and monetary cost. The recovery of pine marten (*Martes martes*) in Britain may provide a natural solution to grey squirrel management, as has been evidenced in Ireland. Pine marten recovery has been supported with successful translocations from Scotland to England and Wales. Here, I will briefly report on the recovery of pine marten in Britain including new habitat suitability models showing important habitats and possible future release sites. I will then present the results of a DNA analysis of pine marten scats from across Britain, indicating the proportion of scats containing grey squirrel and others key species DNA. Finally, I will describe new evidence indicating the effects of predators, pine martens and goshawks (*Accipiter gentilis*), on grey squirrel damage and density.



### Development of a national recovery strategy for red squirrels in England through structured decision making

Smith, Bethany R.<sup>1\*+</sup>; Kenup, Caio F.<sup>1\*+</sup>; Walsh, Katherine<sup>2</sup>; Haw, Kay<sup>3</sup>; Canessa, Stefano<sup>4,5</sup>; Ewen, John G.<sup>1</sup>

- <sup>1</sup> Zoological Society of London, Institute of Zoology, London, UK
- <sup>2</sup> Natural England, UK
- <sup>3</sup> UK Squirrel Accord, UK
- <sup>4</sup> University of Bern, Institute of Ecology and Evolution, Bern, Switzerland
- <sup>5</sup> University of Milan, Department of Environmental Science and Policy, Milan, Italy
- \* bethany.smith@ioz.ac.uk; caio.kenup@ioz.ac.uk
- <sup>+</sup> Authors should be considered as joint first authors with correspondence addressed to both

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Red squirrels (*Sciurus vulgaris*) are an endangered native species in England that face multiple threats including competition with the invasive grey squirrel (*Sciurus carolinensis*), the squirrelpox virus transmitted by grey squirrels, and habitat loss and degradation. Conservation actions are varied with some, primarily grey squirrel management, being less palatable to regional and national communities. Making decisions about how best to conserve red squirrels is, therefore, a complex task.

In this project, we used structured decision making to help develop a national recovery strategy for red squirrels in England. Structured decision making helps to methodically think through difficult decisions and allows for decisions to be informed by scientific evidence alongside the values and risk attitudes of the people involved. Through a series of workshops, we identified the core objectives that people want to achieve with a recovery plan; persistence of red squirrels in England whilst considering public support, animal welfare, wider ecosystem benefits, socioeconomic benefits, and the cost of any management. We then predicted the outcomes of different management strategies with regards to each objective. This included developing a spatially explicit population model of red squirrels and grey squirrels across England to predict the effects of the potential management actions on red squirrel recovery. This model considered how processes such as interspecific competition, squirrelpox epidemiology, and pine marten (Martes martes) predation affect squirrel population dynamics. We also conducted expert elicitation workshops to understand the impacts of each management strategy on squirrel welfare using a modified Sharp & Saunders approach to assessing the welfare implications of controlling pest species. As is common with competing objectives, there was no clear strategy that performed best for each objective. Consequently, we performed a trade-off analysis with the decisionmaker. In this presentation, we will outline the structured decision-making process and its outcomes for this project, offering valuable insights for those tackling the complexities of vertebrate management.



## Implementation of monitoring methods for dormice with emphasis on the forest dormouse (*Dryomys nitedula*) and the hazel dormouse (*Muscardinus avellanarius*) in Croatia

Bjedov, Linda<sup>1\*</sup>; Čuljak, Dora<sup>2</sup>; Huten, Tea<sup>3</sup>; Lugić, Edin<sup>5</sup>; Lisičić, Duje<sup>4</sup>; Boljfetić, Marko<sup>4</sup>; Petković, Monika<sup>2</sup>; Hamidović, Daniela<sup>6</sup>; Kryštufek, Boris<sup>7</sup>; Tvrtković, Nikola<sup>8</sup>

- <sup>1</sup> University of Zagreb, Faculty of Forestry and Wood Technology, Zagreb, Croatia
- <sup>2</sup> Oikon Ltd. Institute of Applied Ecology, Zagreb, Croatia
- <sup>3</sup> Supernatural Ltd., Zagreb, Croatia
- <sup>4</sup> University of Zagreb, Faculty of Science, Zagreb, Croatia
- <sup>5</sup> Elytron, Zagreb, Croatia
- <sup>6</sup> Ministry of Environmental Protection and Green Transition, Institute for Environment and Nature, Zagreb, Croatia
- <sup>7</sup> Slovenian Museum of Natural History, Ljubljana, Slovenia
- <sup>8</sup> Association Natura Society for Nature Protection of Croatia, Zagreb, Croatia

\* lbjedov@sumfak.unizg.hr

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In Croatia, there is a general lack of arboreal small mammal monitoring. This results in a deficiency of population as well as distribution data on arboreal dormice, making strategic planning and conservation measures almost impossible. As part of the Operational Program Competitiveness and Cohesion 2014-2020, the Republic of Croatia implemented a national monitoring program for the forest dormouse (Dryomys nitedula) and the hazel dormouse (Muscardinus avellanarius). Both species were categorized in the last Croatian Red List as Near Threatened (NT) and are included in Appendix IV of the EU Habitat Directive. Croatia is required to report every 6 years on the conservation status of these species. As part of the national monitoring project, various methods were tested and implemented, including live traps, nest boxes, camera trapping, and footprint tunnels. Camera trapping was tested across the entire known and potential distribution ranges for both species in selected 23 quadrants (10x10 km). Cameras were placed on trees with feeding platforms approximately 2 m above the ground and were deployed during the dormice active season from May to September. In each quadrant, ten cameras were placed in a transect, at least 300 m apart, and were recording for the following 10 days. Additionally, all four methods were tested in one guadrant where both dormice species occur, to test their efficiency. Cameras proved to be highly effective in detecting the presence of the target species as well as other two dormice species (Glis glis and Eliomys quercinus) present in Croatia.



# Immigration predictors of wild house mice for potential population management using the *t*-haplotype

Goedecker, Caspar<sup>1\*</sup>; Gonzalez, Ellen<sup>1</sup>; Manser, Andri<sup>1</sup>; Lindholm, Anna K.<sup>1</sup>

<sup>1</sup> University of Zürich, Institute for Evolutionary Biology and Environmental Studies, Zürich, Switzerland \* caspar.goedecker@uzh.ch

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House mice (*Mus musculus domesticus*) are one of the major mammalian pest species worldwide. As an invasive species they cause massive damage to local flora and fauna especially on islands. Half of the large-scale eradication attempts using rodenticides have been unsuccessful so far. The efficiency of rodenticides is limited by the accessibility of the habitat and reduced by the increasing number of species that are developing resistance to rodenticides. In addition, their use is raising major concerns regarding animal welfare including the risk of non-target effects. An alternative approach to managing large, isolated populations are genetic biocontrol methods. One of them could be an inherited sterility method using a selfish genetic element, the so-called *t*-haplotype. Homozygous males carrying this gene are fully sterile while heterozygous males transmit *t* to up to 95% of offspring rather than the expected Mendelian rate of 50% due to gene drive. However, despite the theoretical possibility of such a management method, some key question regarding its implementation remains unanswered. Populations of wild house mice are closed, and the migration of individuals is rare. Therefore, a crucial question is the extent to which animals can be translocated into an existing population, and what individual traits improve translocation success.

In this study, we conducted enclosure experiments on a wild strain of house mice where four animals (*t*-carriers and wildtypes) were released into established populations of ten individuals. We measured the social interactions of released mice with the resident population and gave the animals a possibility to leave the experiment via a water barrier. Immigrants were also tested in behavioural assays for exploration and anxiety. This allowed us to quantify the ability to settle in the population as a function of sex, genotype, and behavioural traits. We found no effect of genotype, but a general advantage of females (67%) over males (11%) in integration into a population. Further, less anxious females were more likely to stay. In general, we found individuals with more social contacts within the first 7 days remained until the end of the experiment. These results suggest that more social and less anxious individuals are more likely to be integrated into an existing population. For this reason, the selection of more social animals could increase the probability for success with such a management method.



# Monitoring olm (*Proteus anguinus*) populations in karstic springs via remote sensing

Valenčič, Meta<sup>1,2\*</sup>; Fišer, Žiga<sup>1</sup>; Kekec, Blaž<sup>1,2</sup>; Premate, Ester<sup>1</sup>; Recknagel, Hans<sup>1</sup>

<sup>1</sup> University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia

<sup>2</sup> Herpetološko društvo – Societas Herpetologica Slovenica, Ljubljana, Slovenia

\* metavalencic@gmail.com

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The olm (*Proteus anguinus*) is one of the largest fully groundwater-adapted vertebrates in the world. This unique amphibian is a Dinaric Karst (Western Balkans) endemic and is threatened by groundwater pollution, habitat destruction, and poaching. Recent phylogenomic analyses revealed the olm is not a single species, but a complex of nine lineages. Some of these are narrow endemics, known only from a few locations, and as such highly endangered: a single manure spill could potentially wipe out an entire lineage. Establishing a monitoring scheme for olms and their groundwater habitat is therefore vital, however not trivial due to the hard-to-access subterranean environment. Karstic springs, on the other hand, are easy-to-access and some olm lineages are known to occur in springs. We assessed the suitability of these windows into the underground and used remote sensing for olm monitoring. We set up camera traps and data loggers (T, light intensity) at three springs in south-eastern Slovenia (Europe), inhabited by two olm lineages. We mounted one camera above water to directly monitor olm presence and a second camera on a nearby tree to monitor spring visitors. Preliminary analysis showed that olm abundance differs throughout the year. At the two natural springs they were active almost exclusively during night-time, while at the artificially covered spring (i.e., a permanently dark environment) they appeared throughout the day. This suggests that light deters olms from using springs. The three springs also differed in olm density and occurrence probability. Moreover, we have detected potential olm predators (e.g. water snakes, red fox, cats), but no predatory behaviour towards the olm has been observed so far. Additionally, we recorded a potential poaching attempt. In conclusion, long-term monitoring could establish spring-specific baseline values of olm density and occurrence probability during different seasons and significant deviations could alarm us of negative population trends. Finally, data gathered hold potential for discovering more on the olms' behaviour and intraspecific as well as interspecific interactions.



# Temporal population genomics reveals evidence of load purging in small, isolated populations of conservation concern

Heenkenda, Erangi J.<sup>1</sup>; Black, Andrew N.<sup>1</sup>; Stockwell, Craig A.<sup>2</sup>; DeWoody, Andrew J.<sup>1,3\*</sup>

<sup>1</sup> Purdue University, Department of Forestry and Natural Resources, West Lafayette, Indiana, USA

<sup>2</sup> North Dakota State University, Department of Biological Sciences, Fargo, North Dakota, USA

<sup>3</sup> Purdue University, Department of Biological Sciences, West Lafayette, Indiana, USA

\* dewoody@purdue.edu

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Small, isolated populations are susceptible to genomic erosion that can lead to declines in individual fitness and population productivity. Nevertheless, freshwater fishes are often found in small, isolated populations as exemplified by desert pupfishes (family Cyprinodontidae) that often exist in extreme isolation. Pupfishes in the Tularosa basin of New Mexico consist of two native populations (Malpais Spring and Salt Creek) as well as two non-native populations (Lost River and Mound Spring) that were established as refugia using founders from Salt Creek. The native populations diverged recently (~4500 years ago) due to genetic drift. We used whole genome sequences from 214 individual pupfish to monitor short-term evolutionary dynamics (~18 generations). By comparing geographic sites and temporal replicates, we monitored patterns of genomic diversity, inbreeding, load and adaptive potential of these endangered pupfishes. Heterozygosity was exceedingly low (~0.0001) and effective population sizes were consistently small (<1000). Nearly one-quarter of the typical genome was autozygous and estimates of genetic load indicate that purging was modest and negatively correlated with  $N_{e}$ . The native sites each harbour distinct gene pools on unique evolutionary trajectories. The refugia sites, which exhibited the lowest genomic diversity, could benefit from reciprocal gene flow with the native Salt Creek population.



# Conservation of capercaillie (*Tetrao urogallus*) in Poland: monitoring the effects of reintroduction and genetic rescue

Santorek, Anna<sup>1</sup>; Rutkowski, Robert<sup>1\*</sup>; Gwiazdowska, Aleksandra<sup>1</sup>; Grabowska, Julia<sup>1</sup>; Merta, Dorota<sup>2</sup>; Ławreszuk, Dorota<sup>3</sup>; Flis-Martyniuk, Ewa<sup>4</sup>

<sup>1</sup> Museum and Institute of Zoology, Warsaw, Poland

<sup>2</sup> Pedagogical University of Cracow, Department of Ecology and Environmental Protection, Cracow, Poland

<sup>3</sup> State Forests, Głęboki Bród Forest District, Głęboki Bród, Poland

<sup>4</sup> State Forests, Janów Lubelski Forest District, Janów Lubelski, Poland

\* rrutkowski@miiz.waw.pl

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Molecular genetic methods, especially those related to the genetic identification of individuals, are extremely useful in monitoring populations of endangered species. The capercaillie (*Tetrao urogallus*) is completely protected in over 20 European countries, and in most of them it is Red-Listed. In Poland, in the second half of the 20th century, the species occurred mainly in the mountains, but survived also in three lowland populations. Unfortunately, one of them –the Lower Silesian Forest– became extinct in the late 1990s, and the other two have experienced a significant decline in number. In the 21<sup>st</sup> century, an attempt was made to actively protect capercaillie in Poland. As part of these activities, the species was reintroduced to the Lower Silesian Forest and the genetic rescue of the two remaining lowland populations, i.e., the Augustów Forest and the Solska Forest, was initiated.

The presented results summarize the effects of over ten years of conservation activities. Based on microsatellite genotyping of non-invasive samples collected in Polish lowland populations, the effectiveness of genetic rescue was determined – the genetic diversity of the populations was compared before and after the reinforcement with reintroduced individuals (Augustowska Forest, Solska Forest). In the case of the extinct population (Lower Silesian Forest), based on microsatellite genotypes of reintroduced birds and an extensive collection of non-invasive samples, the survival and reproductive efficiency of reintroduced individuals were monitored. In total, for genetic analysis more than 1500 samples were collected between 2010 and 2023, including biological material from introduced birds and non-invasive samples found in a field. The results emphasize the importance of active conservation measures to ensure the survival of endangered species populations.



# Before and after: long term monitoring of birds and bats in a wind farm do not show significative impact

Scaravelli, Dino<sup>1,2\*</sup>

<sup>1</sup> University of Bologna, BIGEA Department, Bologna, Italy

<sup>2</sup> STERNA scarl, Forlì, Italy

\* dino.scaravelli@unibo.it

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Careful monitoring of wind farm installation sites is essential to reduce the impacts generated on birds and bats by these plants. A long-term monitoring program was applied in the design and control of a plant of 6 wind turbines with a height of about 80 m at the hub and a rotor with a diameter of 117 m, located on the mountain ridge between the provinces of Parma and Genoa, in the Emilian Apennines. In 2013, the pre-operation monitoring was conducted, which defined the local faunal conditions. After construction in 2018, the monitoring was repeated, with BACI characteristics, extended until 2023. The nesting birds were recorded by singing from points that allowed us to cover the entire extension of the wind farm, and the migratory passages were verified by sight from salient points. The bats were monitored from listening points with classic bioacoustic methods.

The disturbance was significant during the construction phase, but subsequent surveys showed a very rapid recovery of the nesting contingent. The number of nesting species close to the wind farm, considering 1 km of buffer, was 38 in preconstruction phase and 38 in 2018 (i.e., first year post construction), 38 in 2019, 37 in 2020, 43 in 2021, 41 in 2022, and 47 in 2023, respectively. The number of couples slightly increased especially for *Lanius collurio* and *Lullula arborea*, as well as for the forest dwelling species. The migratory passage at the wind farm direction is modest, with a good diversity with 78 species in 2023, 69 in 2022, 75 in 2021, 74 in 2020, 59 in 2019, and 60 in 2018, also including local movements that are not typically migratory. The number of passages for species of conservation interest (i.e., those included in the national and European lists) was 338 individuals in 2023, 332 in 2022, 260 in 2021, 222 in 2020, 198 in 2019, and 230 in 2018, both regarding own migrants and local passage or birds present in the area. The checks in the pitches to verify fatalities were carried out four times a month, without ever finding any carcasses remained on the ground for 1 to 3 days before consumption.

As regards bats, 6 species were counted and the average number of passages per hour did not undergo significant variations in the years of observation, increasing progressively from the beginning of spring with a peak in the warm months, with the presence of many insects in open areas, then decreasing and finally interrupting with the cold of October.

For the site under monitoring, it is possible to conclude that at such a choice of area, the spatial arrangement of the towers and the management of open spaces throughout the farm, this wind farm did not have a negative impact on studied taxa; indeed, it seems that it even facilitated some indicator species.



### Nocturnal raptors for vole control: studying their populations

Cuéllar-Basterrechea, Carlos<sup>1\*</sup>; Hernández-Garavís, Lorena<sup>1\*</sup>; Báscones-Reina, Miriam<sup>1</sup>; Garcés-Toledano, José Fernando<sup>1</sup>; Blanca-Chana, Fernando<sup>1</sup>; Rodríguez-Moreno, Beatriz<sup>1</sup>; González-Simón, Paula<sup>1</sup>; Peiteado-Morales, Celsa<sup>2</sup>

<sup>1</sup> Group for the Rehabilitation of Native Fauna and its Habitat (GREFA), Majadahonda, Spain

- <sup>2</sup> World Wildlife Found Spain (WWF/España), Madrid, Spain
- \* carlos@grefa.org

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Common vole (*Microtus arvalis*) population outbreaks generate important damage in croplands of northwest of Spain. In order to work on the control of this pest, a study area was established in the province of Segovia. The area was equipped with 103 nest boxes for avian predators: barn owl (*Tyto alba*), little owl (*Athene noctua*), and common kestrel (*Falco tinnunculus*). Literature and phototrapping have previously demonstrated that these two owl species are effective predators of common vole. They both have a poor conservation status in Spain.

A specific monitoring study was developed in the period 2021-2024, to individualize all the nocturnal raptors which breed in these nest boxes. The goal was to get knowledge and demonstrate the importance of fixing populations of these raptor species in croplands where rodents frequently cause damage. The study demonstrated that it is possible to recover and fix populations of nocturnal raptors for a period of many years. This requires the installation of a considerable number of nest boxes to offer enough breeding sites for future generations of owls. Thanks to this inexpensive tool, we have obtained important information on the kinship, breeding pairs, local movements, and habitat selection of two main feathered allies of Spanish farmers: barn owl and little owl. This information is very useful to know how to orientate new projects or local actions of biological control of the common vole. Finally, we have shown that our monitoring method poses no risk to these birds at such a delicate time as the breeding season.



## Lessons learnt from thirty years journey of ecologically based rodent management system in Asia

Htwe, Nyo Me<sup>1\*</sup>; Singleton, Grant R.<sup>2</sup>; Sudarmaji, Sudarmaji<sup>3</sup>; Jacob, Jens<sup>1</sup>

- <sup>2</sup> University of Greenwich, Natural Resources Institute, Kent, UK
- <sup>3</sup> National Research and Innovation Agency (BRIN), Cibinong Science Center, Research Center for Food Crops, Bogor, West Java, Indonesia
- \* nyo.me.htwe@julius-kuehn.de

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Rodents are important pests to agriculture in Asia, particularly to the staple cereal crop of rice. Importantly, the losses caused by rodents can have a substantial impact on food security of rural households. In Asia, ecologically based rodent management (EBRM) was first introduced in Indonesia following research on the ecology of the main pest species, *Rattus argentiventer*, and the factors influencing its population dynamics. Large scale EBRM has since been implemented in Vietnam, Laos, Philippines, Myanmar, Cambodia, Bangladesh, and China. EBRM components in different countries differ based on the key rodent species, cropping system, and landscape. Sustainable adoption in the community after the project ends is a considerable challenge in developing countries.

Here, we present studies from the lowland irrigated system in Indonesia and upland agriculture system in Myanmar to analyse the process and success of EBRM. An adaptive research management (AD) approach was applied in both countries, which promotes greater community adoption as well as rapid technology diffusion to the farming communities. We found that the extent of adoption depends on the farmers' priority and commitment towards rodent management (based on the tolerable level of losses caused by rodents), the interest of policy makers, and the attitude of farmers in working together as a community. In Myanmar and Indonesia, the rapid adoption (within 1-2 years) of AD-EBRM promoted an increase in rice production from 12-75%. A key lesson learned from our long-term EBRM journey in Asia was that all stakeholders need to work together harmoniously if EBRM is to be implemented successfully on a large-scale. An important foundation of outreach is the certification of EBRM as a national policy for rodent management in agricultural systems.



<sup>&</sup>lt;sup>1</sup> Julius Kühn Institute (JKI), Federal Research Institute for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Rodent Research, Münster, Germany

#### Invasive species management strategies in a One Health system

August, Katherine<sup>1\*</sup>; Beatham, Sarah<sup>2</sup>; Birtles, Richard<sup>3</sup>; Bray, Daniel<sup>1</sup>; Broadhurst, Holly<sup>3</sup>; Croft, Simon<sup>2</sup>; Marshall, Harry<sup>4</sup>; Massei, Giovanna<sup>5</sup>; Orchard, Rachel<sup>4</sup>; Parkes-Withers, Amy<sup>2</sup>; Belmain, Steven<sup>1</sup>

- <sup>1</sup> University of Greenwich, Natural Resources Institute, Medway, UK
- <sup>2</sup> Animal and Plant Health Agency, York, UK
- <sup>3</sup> University of Salford, School of Science, Engineering & Environment, Salford, UK
- <sup>4</sup> Forest Research, Edinburgh, UK
- <sup>5</sup> University of York, Department of Environment & Geography, York, UK
- \* katie.august@greenwich.ac.uk

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Grey squirrel (*Sciurus carolinensis*) is an invasive species in the UK which threatens plants through bark stripping and wildlife through disease transmission and competition. Grey squirrels are also of growing relevance to human health as reservoir hosts of both *Borrelia burgdorferi*, the agent of Lyme disease, and *Ixodes ricinus*, the tick vector which feeds on a range of animal species and people. Current strategies to manage grey squirrel populations require intense trapping and/or shooting. The potential of fertility control to reduce grey squirrel populations has not been tested. While fertility control may have a slower impact on population density, it would alter the age ratio in the population to reduce numbers of juvenile squirrels which are more likely to disperse and transmit pathogens and ticks than adults. In this project, we are comparing the potential efficacy of fertility and traditional control methods in isolated woodlands in Cumbria, UK. We estimated grey squirrel and tick densities before and after three culling treatments: no cull, full traditional cull, and a simulated fertility treatment cull whereby only juvenile squirrels are removed from the population. Ticks will be tested for pathogen presence and blood meal analysis performed to assess the host species being fed upon. Based on the results we will model the effectiveness of fertility control and culling in reducing the risk of Lyme's disease.



### Using novel methods to prioritise grey squirrel control

Ham, Cally1\*; Scopes, Ellie2; Pickering, Mark3; Gill, Robin1

<sup>1</sup> Forest Research, Alice Holt Lodge, Farnham, UK

<sup>2</sup> Forest Research, Forestry Commission National Office, Bristol, UK

<sup>3</sup> University of Southampton, Southampton, UK

\* cally.ham@forestresearch.gov.uk

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Grey squirrel (*Sciurus carolinensis*) is a non-native, invasive species threatening the health, resilience and economic viability of broadleaf woodlands throughout England, Wales, and southern Scotland. The impacts of grey squirrel bark-stripping is estimated to cost £37 million per year in lost timber quality and reduced carbon sequestration with unquantified damage to native biodiversity and woodland function. To date, the only method of controlling grey squirrel damage within woodlands is through lethal population control which is expensive and time consuming. Due to grey squirrel high fecundity and mobility the population is quick to recover following culls through migration and increased birth rate. As squirrel control resources are limited, there is a need for woodland managers to prioritise areas to target their efforts.

We present two novel methods that could aid prioritisation of squirrel control. Firstly, the use of UAVbased sensors to locate and identify signs of canopy damage during the summer season, when groundbased damage assessments are difficult due to leaf cover. And secondly, the use of predictive models to identify when food availability, in the form of European beech (*Fagus sylvatica*) seed, might be high and therefore enable an increase in squirrel population density. We found that UAV-based sensors can detect signals of canopy damage and that these can be identified using machine learning processes. We also found that there is a detectable increase in squirrel numbers recorded in the year following high beech seed availability. Future work will focus on developing a predictive tool to be used by forest managers to identify when and where to target their squirrel control efforts.



# Balancing control methods for invasive tree squirrels with lethal and non-lethal approaches

Bisi, Francesco<sup>1\*</sup>; Santicchia, Francesca<sup>1</sup>; Panzeri, Mattia<sup>1</sup>; Tranquillo, Claudia<sup>1</sup>; Panebianco, Matteo<sup>1</sup>; Sclip, Alessandro<sup>1</sup>; Grespan, Pietro<sup>1</sup>; Tortini, Alessia<sup>1</sup>; Bertolino, Sandro<sup>2</sup>; La Morgia, Valentina<sup>3</sup>; Paoloni, Daniele<sup>4</sup>; Marsan, Andrea<sup>5</sup>; Preatoni, Damiano<sup>1</sup>; Wauters, Lucas<sup>1</sup>; Martinoli, Adriano<sup>1</sup>; Mazzamuto, Maria Vittoria<sup>2</sup>

<sup>1</sup> Università degli Studi dell'Insubria, Department of Theoretical and Applied Sciences, Varese, Italy

- <sup>2</sup> Università degli Studi di Torino, Dipartimento di Scienze della Vita e Biologia dei Sistemi, Torino, Italy
- <sup>3</sup> Istituto Superiore per la Protezione e la Ricerca Ambientale, Ozzano dell'Emilia, Bologna, Italy
- <sup>4</sup> Istituto Oikos srl Impresa sociale, Perugia, Italy
- <sup>4</sup> Universita` di Genova, Department of Earth, Environmental and Life Sciences, Genova, Italy
- \* francesco.bisi@uninsubria.it

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Invasive alien squirrels, particularly the Eastern grey squirrel (Sciurus carolinensis) and Pallas's squirrel (Callosciurus erythraeus), are significantly impacting Italy's ecosystems, threatening native species such as the Eurasian red squirrel (Sciurus vulgaris) through competition for trophic resources and habitat. In southern Italy, the variable squirrel (C. finlaysonii) is also spreading and likely beginning to overlap with the endemic S. meridionalis. In response, Italy has implemented diverse control strategies across different regions aligned with EU Regulation 1143/2014 to curb the spread of these invasive species. In urban parks, small populations have been managed successfully through capture, sterilization, and release (Liguria, western Italy), while urban to rural populations have been controlled using trapping and removal methods (Umbria, central Italy). Early intervention in these regions has led to effective eradication. In areas with larger, more established populations, such as in Piedmont and parts of Lombardy, north-west Italy, lethal removal methods involving capture and euthanasia with CO<sub>2</sub> have been employed. A control plan based on the same methods is currently underway to manage the variable squirrel in parts of its range in Basilicata (southern Italy). Additionally, at the Italian-Swiss border, the population of Pallas's squirrel is being managed using a combination of trapping and culling as part of an integrated strategy. Integrated strategies combining varying degrees of three main removal methods—capture followed by euthanasia, capture followed by surgical sterilization, and culling—are also being adopted to manage and potentially eradicate new grey squirrel outbreaks following early warning reports scattered throughout Italy.

These efforts demonstrate that early and continuous intervention is key to successful eradication, while delayed or sporadic actions are bound to fail, having escalating costs and impact on both biodiversity and financial resources. The Italian case highlights the necessity of a multifaceted approach to invasive squirrel management to safeguard native biodiversity and protect vulnerable native populations.



## Distribution, social perception and potential range expansion of invasive raccoons in Italy

Mori, Emiliano<sup>1,2\*</sup>; Paletto, Alessandro<sup>3</sup>; Ancillotto, Leonardo<sup>1,2</sup>; Lagrotteria, Alessandro<sup>1,4</sup>; De Meo, Isabella<sup>5</sup>; Viviano, Andrea<sup>1</sup>; Simoncini, Andrea<sup>6</sup>; Menchetti, Mattia<sup>7</sup>; Mazza, Giuseppe<sup>8</sup>; Falaschi, Mattia<sup>6</sup>

<sup>1</sup> Istituto di Ricerca sugli Ecosistemi Terrestri IRET, Consiglio Nazionale delle Ricerche, Florence, Italy

<sup>2</sup> National Biodiversity Future Center, Palermo, Italy

- <sup>3</sup> Research Centre for Forestry and Wood, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Trento, Italy
- <sup>4</sup> University of Turin, Department of Life Sciences and Systems Biology, Turin, Italy
- <sup>5</sup> Research Centre for Agriculture and Environment, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Firenze, Italy
- <sup>6</sup> Università degli Studi di Milano, Dipartimento di Scienze e Politiche Ambientali, Milan, Italy
- <sup>7</sup> CSIC-Univ. Pompeu Fabra, Institut de Biologia Evolutiva, Barcelona, Spain

<sup>8</sup> CREA Research Centre for Plant Protection and Certification (CREA-DC), Cascine del Riccio, Florence, Italy

\* emiliano.mori@cnr.it

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Invasive alien species listed in the Annex of the EU Regulation 1143/2014 should be rapidly detected and, whenever possible, eradicated, given their strong impacts on native biodiversity, ecosystems, and health. However, assessing distribution, social perception towards these species and possible corridors for range expansion is decisive for successful management actions. Amongst invasive species of European concern, the Northern raccoon (*Procyon lotor*) has been present with a free-ranging population in central Italy since 2013, following the escape of some individuals from an enclosed area. Since then, the presence of these animals has persisted outside the protected area. The unmistakable characteristics of raccoon, such as its distinctive face mask and ring-tail pattern, make it highly recognizable and highly recorded on citizen-science platforms.

We used a citizen-science, photo-based approach to redefine the distribution of the Northern raccoon in Italy and we developed a standardised questionnaire to test for social perception towards this species. For a management proposal, habitat suitability and connectivity models were run to unveil the expansion history of this invasive species. We collected 61 raccoon occurrences, showing a remarkable range expansion of the species in central Italy, mostly southwards with respect to the introduction site. Moreover, we detected a high public appreciation on over 1000 questionnaires, which highlights the importance of a good communication campaign to enhance the success of removal programmes. To conclude, we showed a high environmental suitability for the raccoon in Europe, which is in line with its generalist behaviour in terms of habitat use. These results underscore the importance of implementing comprehensive removal programs and public outreach initiatives to mitigate the negative impacts of this invasive species.



## Presence, distribution and potential spreading of the invasive alien species, raccoon (*Procyon lotor*), in Tuscany, in relation to environmental characteristics at different spatial scales

Martini, Matilde<sup>1\*</sup>; Tuti, Marco<sup>1</sup>; Ghinassi, Giulia<sup>1</sup>; Cappai, Nadia<sup>2</sup>; Franco, Alessandro<sup>3</sup>; Zaccaroni, Marco<sup>4</sup>; Ponzetta, Maria Paola<sup>1</sup>; Ferretti, Marco<sup>5</sup>; Mattioli, Luca<sup>5</sup>; Romeo, Giorgia<sup>5</sup>; Mazzarone, Vito<sup>5</sup>; Lenuzza, Andrea<sup>5</sup>; Merli, Federico<sup>5</sup>; Bertolucci, Antonio<sup>5</sup>; Panicucci, Alberto<sup>5</sup>; Muzzi, Roberta<sup>5</sup>; Conti, Leonardo<sup>1</sup>; Becciolini, Valentina<sup>1</sup>; Sottili, Caterina<sup>1</sup>; Santini, Giacomo<sup>4</sup>; Bozzi, Riccardo<sup>1</sup>

- <sup>1</sup> University of Florence, Department of Agriculture, Food, Environment and Forestry (DAGRI), Florence, Italy
- <sup>2</sup> Foreste Casentinesi, Monte Falterona e Campigna National Park, Pratovecchio Stia, Italy
- <sup>3</sup> Biologist freelance, Italy
- <sup>4</sup> University of Florence, Department of Biology, Florence, Italy
- <sup>5</sup> Tuscany Region, Wildlife Department, Florence, Italy
- \* matilde.martini@unifi.it

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Invasive alien species (IAS) are a major global threat to ecosystems, disrupting biodiversity and ecosystem functions and contributing significantly to biodiversity loss. Human activities have driven the spread of species beyond their native ranges, increasing introductions worldwide. Raccoon (*Procyon lotor*), an opportunistic mesopredator native to Central America, the United States, and southern Canada, was introduced to Tuscany through the accidental release from a local zoo, near the border of Foreste Casentinesi National Park. Known for its adaptability, raccoon can modify its behaviour and diet in response to local conditions, enabling it to thrive in various habitats, from human-modified landscapes to natural areas. In Tuscany, it has established a stable population, with predation as the primary impact on native species.

Our study aimed to assess the influence of landscape and fine-scale characteristics on raccoon distribution in Tuscany and create dispersal maps to forecast potential expansion into nearby regions. According to the Italian National Management Plan of the Raccoon, we stratified our study area based on proximity to water sources. We divided the territory into 2.5 x 2.5 km cells and randomly selected trap points along streams or rivers within each cell. Camera traps were deployed starting in June 2024, with sampling planned to continue through 2025. Landscape characteristics were calculated within a buffer around each sampling point, an area equivalent to the average home range size observed for male raccoons from data collected outside their native range. Similarly, fine-scale characteristics were assessed within a buffer corresponding to an average size of male raccoon core area. We will present results from the first year of monitoring, providing a comprehensive analysis of the ecological factors that shape raccoon distribution, allowing us to explore how landscape features and habitat characteristics influence their presence and potential expansion in Tuscany. By assessing these dynamics, we can better provide the species' likelihood of colonizing new areas and better manage its spread in non-native areas.



# Sacred or unholy? Assessing the impact of sacred ibis (*Threskiornis aethiopicus*) on heronries in northern Italy

Larroux, Nicola<sup>1\*</sup>; Gagliardi, Alessandra<sup>2</sup>; Volponi, Stefano<sup>3</sup>; Tenan, Simone<sup>4</sup>; Preatoni, Damiano<sup>1</sup>; Martinoli, Adriano<sup>1</sup>

<sup>1</sup> Università degli Studi dell'Insubria, Department of Theoretical and Applied Sciences, Varese, Italy

<sup>2</sup> Istituto Oikos ETS, Italy

<sup>3</sup> Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Italy

<sup>4</sup> National Research Council, Institute of Marine Sciences (CNR-ISMAR), Italy

\* nlarroux@studenti.uninsubria.it

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Sacred ibis (*Threskiornis aethiopicus*) has been listed as an invasive alien species of European concern since 2016. Despite clear management responsibilities, actual knowledge of its impacts is still dubious and fragmented. Due to delayed management measures adopted in Italy, sacred ibis populations have grown largely undisturbed since the 2000s, now providing a unique opportunity to examine the consequences beyond the early stages of invasion. Breeding ecology of the species in the Italian nesting core area (Po plain) has been studied. Moreover, interactions with the native fauna were assessed within the mixed heronries colonies, where sacred ibis breeds in proximity of protected species, through the use of various monitoring techniques.

Direct observations and camera trap footage show that sacred ibis has no interest in preying neither eggs nor nestlings of neighbouring colonial species. Experiments with artificial nests containing various egg types provided analogous results. Sacred ibis displayed only partially hostile behaviour, limited to stealing nest material from nearby herons. Additionally, ground-nesting sacred ibises suffered high levels of predation by other birds (e.g. jackdaw, herring gull) and mammals (e.g. red fox, European badger). Overall, this study does not support the alleged role of sacred ibis as an invasive predator, which has been however proven elsewhere (France).



# **Optimizing urban rodent control strategies: predictive approach and risk assessment – the contribution of SECU-RAT**

Sage, Mickaël<sup>1,2\*</sup>; Riehling, Philippe<sup>1</sup>; Hoffmann, Julien<sup>1</sup>

<sup>1</sup> SECU-RAT & Faune INNOV' R&D, Besançon, France

<sup>2</sup> Faune INNOV' R&D, Besançon, France

\* m.sage@secu-rat.fr

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Rodents pose a significant problem to society (e.g. zoonoses, severe property damage, food loss). Urban environment favours high population densities, especially of brown rats (*Rattus norvegicus*). Increasingly high infestation levels are observed, in particular where there is a high human presence (e.g. urban parks, along riverbanks, where birds are fed, on picnic spots). Rodent population control is mainly based on the use of rodenticides in bait boxes and trapping. However, due to high food availability and the presence of attractive habitats in these areas, rat population control is particularly difficult and often ineffective. After decades of rodent control, they continue to persist in cities around the globe and a new approach is needed. Placing traps or rodenticides close to rat burrows, habitats, or food sources is essential, but the discretion requirement, bait box degradation risk, and children and non-target species safety are major issues linked to these practices. Public bins are of major concern because they offer food to rats, but also because they fit into the urban outdoor environment and are often placed near rat habitats such as flowerbeds, bushes, etc.

SECU-RAT addresses two major challenges: (i) ensuring the highly effective and secure deployment of rodenticide baits and trap in the areas exactly where they are most needed in an urban outdoor environment: under public bins; (ii) offering a new comprehensive and integrative tool for decision support in the monitoring and control of rodents in urban environments.

Resulting from academic research, our work aims to develop an innovative approach for more effective management of rodent populations addressing health, material, economic, environmental, and ethical concerns. Based on a range of sensors for detecting the presence and trapping of rodents, our decision-making tool enables the population to be monitored in real-time, data analysis, automated reports and alerts, high-stakes areas mapping, identifying and anticipating health issues for risk assessment, optimized decision-making, effectiveness tracking of interventions.

Our approach based on the superimposition of different GIS layers will be presented: favourable habitats (e.g. sewer system, green spaces), available food resources (e.g. litter bins, composters, food shops), rat "densities" measured with SECU-RAT, and human activities at risk (e.g. human density, children's play areas, restaurant terraces). This new comprehensive tool represents a significant advancement in sustainably mitigating the impact of rodents in urban areas. It combines advanced technology with integrated management under the One Health approach promoting a healthier and safer environment.



## Using a naturally occurring sterility gene to control invasive house mice

Manser, Andri<sup>1\*</sup>; Lindholm, Anna K.<sup>1</sup>

<sup>1</sup> University of Zürich, Institute for Evolutionary Biology and Environmental Studies, Zürich, Switzerland \* andri.manser@ieu.uzh.ch

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House mice (*Mus musculus*) are a major ecosystem pest with devastating impact on island ecosystems as non-native invasive species. Current control methods — primarily aerial broadcasting of anticoagulant poisons— raise serious concerns about animal welfare, cause off-target impacts on other species, and are challenging to use in human-inhabited areas. Our research investigates an alternative control strategy using a naturally occurring genetic variant in mice, known as the *t*-haplotype, as a humane and targeted alternative for mouse population management.

The *t*-haplotype has two promising characteristics for pest control. First, males that carry two copies of the gene (t/t homozygotes) are completely sterile. Similar to sterile insect techniques, releasing these sterile males into target populations could reduce reproductive rates by increasing the frequency of unproductive mating. Second, males carrying only one copy (+/t heterozygotes) transmit the t to more than 90% of their offspring instead of the 50% expected under Mendelian inheritance. Thanks to this super-Mendelian mode of inheritance, also known as gene drive, we would expect the t-haplotype to spread genetically throughout a target population despite its (sterility) cost, further increasing the frequency of sterile males, potentially leading to population decline.

Here, a mathematical model assessing the potential of the sterile *t*-method for mouse population control will be presented. Our results indicate that eradication is theoretically achievable based on current knowledge. However, the feasibility critically depends on several biological factors that are currently unknown and that require future empirical work, such as population demography, mating behaviour, and the ability of released males to settle and establish target populations. The model further helps to identify strategies to optimise release campaigns. If viable, this approach could offer a safe, effective, humane, and GMO-free alternative to conventional mouse control methods.



## New regulatory toxicology and field efficacy advances to support the registration of norbormide

Eason, Charles<sup>1\*</sup>; Shapiro, Lee<sup>2</sup>; MacMorran, Duncan<sup>3</sup>; Rennison, Dave<sup>4</sup>; Smith, Karl<sup>5</sup>; Ogilvie, Shaun<sup>6</sup>; Brimble, Margaret<sup>4</sup>

- <sup>1</sup> Lincoln University, Faculty of Agriculture and Life Sciences, Centre for Wildlife Management and Conservation, Lincoln, New Zealand
- <sup>2</sup> Boffa Miskell Ltd, Auckland, New Zealand
- <sup>3</sup> IPC Ltd, Auckland, New Zealand
- <sup>4</sup> University of Auckland, Auckland, New Zealand
- <sup>5</sup> TCC (NZ) Ltd, Auckland, New Zealand
- <sup>6</sup> University of Canterbury, Christchurch, New Zealand
- \* Charles.Eason@lincoln.ac.nz

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To develop a new or re-register an older rodenticide a large financial investment and commitment is required similar to that occurred recently in Europe with cholecalciferol. Norbormide has unique attributes, being 100 to 150-fold more toxic to rats than to birds and most other mammals, and therefore also justifies this investment. Differences in the effects of norbormide, and its selective contractile effect on rat peripheral arteries and the lack of an effect on the peripheral or small blood vessels in other species appear to be responsible for species differences in toxicity. This understanding of the mechanism of toxicity in rats and species-specific changes is complemented by molecular toxicology explaining why this compound has an effect that is rapid, irreversible, and unique to rats.

There is an extensive database in over 50 species with most of the publications circa 1965. These studies have not been carried out in accordance with recent test guidelines. Standard Organization for Economic Cooperation and Development (OECD) guideline studies are being completed to focus on data-gaps in chemistry, genetic toxicology, non-target toxicity, general ecotoxicity, and environmental fate. To date norbormide has been shown to lack genotoxicity in OECD 477, 487 and 490, *in-vitro* studies and it lacked irritancy in OECD 404, 406 and 407. Further OECD studies are yielding results consistent with historical data that reported LD50 ranges, following oral administration for Norway rats (*Rattus norvegicus*), between 5.3 and 15.0 mg/kg. The lack of toxicity (i.e., LD50 > 1,000 mg/kg) is previously reported in five bird species and numerous mammalian species, including rhesus monkeys.

Inconsistencies in palatability hampered norbormide use when it was first developed. Prior to embarking on new chemistry and toxicology OECD studies over a decade of research which focused on synthetic processes yielded a manufacturing approach which consistently produced palatable and effective norbormide. This has enabled consistent efficacy of 1% norbormide-containing baits in laboratory and field trials with both Norway rats and black rats (*Rattus rattus*). Details on new developments including results from the current programme of testing, ranging from the Ames test to non-target testing in birds and earthworms, addressing new data requirements or verification of early data to enable registration, will be presented.



### Monitoring rodenticide exposure in live carnivores

Quinn, Niamh<sup>1\*</sup>; Day, Carolyn<sup>1,2</sup>; Stapp, Paul<sup>2</sup>

<sup>1</sup> University of California, Agriculture and Natural Resources, Irvine, USA

<sup>2</sup> California State University, Fullerton, USA

\* nmquinn@ucanr.edu

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Anticoagulant rodenticides have been detected in many species of wildlife worldwide. In California, anticoagulant rodenticides are considered to be of high regulatory concern and are considered highrisk for wildlife. The current monitoring system that provides information on rodenticide exposure to the Department of Pesticide Regulation (DPR) has a number of limitations. Only sick, injured or dead animals are examined, so the overall prevalence of exposure cannot be determined at the population level. A proposed system of monitoring will be discussed that will provide DPR with an index of the rate of exposure over time from the proportion of positive animals out of the total number of animals tested from wild populations. The samples were collected haphazardly from free-roaming, live individuals in the wild and, for the first time, the monitoring does not rely on samples from sick or injured animals or carcasses.



## DEERplex: New genotyping-by-sequencing STR markers for noninvasive genetic monitoring of deer populations and application in Romanian Carpathians

Konec, Marjeta<sup>1,2\*</sup>; Pazhenkova, Elena<sup>2</sup>; Pavlovič, Eva<sup>2</sup>; Boyer, Frédéric<sup>3</sup>; De Barba, Marta<sup>1,2</sup>; Iosif, Ruben<sup>4</sup>; Promberger-Füerpass, Barbara<sup>4</sup>; Miholcea, Zsolt Levente<sup>4</sup>; Boljte, Barbara<sup>1</sup>; Hrga, Nuša<sup>1</sup>; Skrbinšek, Tomaž<sup>1,2</sup>

<sup>1</sup> DivjaLabs Ltd., Ljubljana, Slovenia

- <sup>2</sup> University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
- <sup>3</sup> Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, Laboratoire d'Ecologie Alpin, Grenoble, France
- <sup>4</sup> Foundation Conservation Carpathia, Braşov, Romania
- \* marjeta.konec@bf.uni-lj.si

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Over the last couple of decades, genetic tools have become indispensable for providing monitoring data for research and management of wildlife. However, practical applications were often limited by the considerable drawbacks of the traditionally used microsatellites (STR) analysed using capillary electrophoresis. A degree of subjectivity in allele calling, difficulties in data sharing between laboratories and slow, labour intensive, expensive analyses have particularly hampered their use in large, managed populations of large herbivores. This is particularly true for red deer (*Cervus elaphus*): despite the considerable management interest, population-level studies using noninvasive genetic sampling are still rare. Typically, thousands of samples would need to be analysed, making such studies slow and expensive with the traditional approaches. We aimed to overcome this by developing and testing a new genotyping-by-sequencing marker system. Building on successful applications of the method for large carnivores, we developed a new set of high throughput sequencing (HTS) markers for red deer genotyping.

Using a bioinformatic pipeline and existing red deer genomes, we designed 181 STR markers and five sex-ID markers. By dividing them into several multiplexes, we tested the performance of primers and their polymorphism directly in noninvasive samples. In the next step we selected 46 best performing STR markers and kept 4 sex-ID markers. By optimizing the protocol for practical application, we produced a final single multiplex of 15 STR markers and 2 sex-ID markers. This protocol can be readily scaled-up and automated in the laboratory. As allele calling is done through bioinformatic analysis and requires only limited manual checking, the approach allows cost-effective, rapid genotyping of thousands of samples. Since all data is at the level of nucleotide sequence, the genotypes are perfectly compatible between different laboratories and fully future proof. We tested the approach on 63 red deer faeces samples collected in 2023 in Romanian Carpathians and achieved an 88% genotyping success rate with reliable individual identification. Encouraged by these results we expanded sampling efforts in 2024, analysing 506 additional samples. Our method provides a robust, reliable foundation for population size, density estimates and parentage analyses in red deer, opening the door for largescale genetic monitoring of this large herbivore. The approach that allowed us to rapidly develop and optimize an HTS genotyping system can be easily applied to other species, providing new possibilities for transborder monitoring and management of large herbivore populations.



# iDeer: An integrated decision-support tool for managing deer alongside woodland creation

Gresham, Amy<sup>1,2\*</sup>; Grainger, Matthew<sup>3</sup>; St John, Freya<sup>4</sup>; Cini, Elena<sup>4,5</sup>; Eigenbrod, Felix<sup>2</sup>; Bellamy, Chloe C.<sup>6</sup>; Guy, Matt<sup>7</sup>; Rattey, Andrew<sup>6</sup>; Ward, Alastair<sup>8</sup>; Shannon, Graeme<sup>3,4</sup>; Spake, Becks<sup>1,2</sup>

<sup>1</sup> University of Reading, School of Biological Sciences, Reading, UK

<sup>2</sup> University of Southampton, Institute for Life Sciences, Southampton, UK

<sup>3</sup> Norwegian Institute for Nature Research, Trondheim, Norway

- <sup>4</sup> Bangor University, School of Environmental and Natural Sciences, Bangor, UK
- <sup>5</sup> Anglia Ruskin University, Global Sustainability Institute, Cambridge, UK
- <sup>6</sup> Forest Research, Northern Research Station, Roslin, UK
- <sup>7</sup> Forest Research, Alice Holt Lodge, Farnham, UK
- <sup>8</sup> University of Leeds, Faculty of Biological Sciences, Leeds, UK
- \* amygresham48@gmail.com

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Major land use transitions associated with Britain's woodland creation schemes are set to accelerate further increases in wild deer densities and distributions, threatening treescape expansion, resilience, and Net Zero targets. Managing deer populations and designing planting schemes to mitigate their impacts constitutes one of the most complex wildlife management challenges across the temperate zone. The iDeer project, funded by the UKRI Future of UK Treescapes programme, is delivering a co-designed, web-based decision support tool that will quantify and map the risk of deer impacts to new and existing woodlands at the landscape scale as a function of alternate planting scenarios. To map predicted risk of deer impacts to individual woodland parcels across England and Wales, we have worked with deer experts to develop predictive Bayesian Belief Network models that incorporate complex interactions among landscape-level factors including forage quality, connectivity, climatic harshness, and human disturbance. The model underpins an interactive, web-based 'iDeer Tool' to predict risk as a function of different woodland planting scenarios inputted by users.



# New methods for testing the effectiveness of wildlife-vehicle collision mitigation measures

Bíl, Michal<sup>1\*</sup>; Andrášik, Richard<sup>1</sup>; Cícha, Vojtěch<sup>1</sup>

<sup>1</sup> CDV – Transport Research Centre, Department of Geoinformatics, Brno, Czech Republic

\* michal.bil@cdv.cz

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Mitigation measures against wildlife-vehicle collisions (WVC) on transportation infrastructure can be categorized into three types, depending on the target group they affect. The first group comprises animals, where measures aim to prevent them from entering roads, particularly in areas with a high risk of collision. For secondary roads, it is advisable to deter or appropriately warn wildlife from crossing. The second group includes drivers, who, on unfenced roads, must remain vigilant during critical periods of the day when wildlife may enter the roadway. The third group involves management practices, encompassing both road infrastructure management and the surrounding landscape. We focus on the first group: animals, and on testing mitigation measures designed for secondary roads. How can we assess the effectiveness of these measures? It is reasonable to expect that effective measures will reduce the number of collisions, and that this reduction will be significant. The common approach involves studies based on monitoring the number of animal carcasses found before and after the installation of measures, including observations on control sections. However, a limitation of such studies is the need to wait until a minimum number of casualties accumulate, which can take a long time. As a result, these studies are time-consuming and therefore costly.

Aware of the limitations of traditional studies, we have focused on monitoring animal movements and behaviour in areas close to the implemented measures. When studying behaviour, it is not necessary to install many mitigation measures, but rather it is crucial to carefully evaluate the movements of the animals. There are two approaches available: the first is to use GPS collar data, though these are usually used for other purposes. The second approach is video analysis. GPS collars are frequently used for tracking the home range or migration/dispersion of wild animals. Due to the limited battery capacity of the devices, GPS positions (fixes) are often recorded at relatively long intervals. However, our objective was to reconstruct the animal's movement trajectory as accurately as possible using a mathematical model. Camera recordings must comprehensively capture the area where the mitigation measures are installed and, crucially, must record the presence of the animal. Since ungulate activity peaks during low-visibility periods, cameras with infrared illumination or thermal cameras are required. In addition to this, it is necessary to identify the animal's movement and, ideally, its behaviour. Given that manual image analysis is time-consuming, we focused on leveraging artificial intelligence methods, specifically image analysis, to automate this process. We will demonstrate how both methods-GPS tracking and image analysis—can be applied to test the effectiveness of mitigation measures and to observe animal behaviour near roads.



## The observatory approach: continental-scale wildlife density estimation in Europe and more

Blanco-Aguiar, J.A.<sup>1\*</sup>; Guerrasio, T.<sup>1</sup>; Carniato, D.<sup>1</sup>; Acevedo, P.<sup>1</sup>; Apollonio, M.<sup>1</sup>; Arakelyan, M.<sup>1</sup>; Arnon, A.<sup>1</sup>; Beatham, S.<sup>1</sup>; Belova, O.<sup>1</sup>; Berde, L.<sup>1</sup>; Berdión, O.<sup>1</sup>; Bleier, N.<sup>1</sup>; Burgui Oltra, J.M.<sup>1</sup>; Bužan, E.<sup>1</sup>; Carro, F.<sup>1</sup>; Carvalho, J.<sup>1</sup>; Casaer, J.<sup>1</sup>; Colomer, J.<sup>1</sup>; Dijkhuis, L.<sup>1</sup>; Duniš, L.<sup>1</sup>; Ertürk, A.<sup>1</sup>; Dal Mas, M.<sup>1</sup>; Ferroglio, E.<sup>1</sup>; Forti, A.<sup>1</sup>; Gačić, D.<sup>1</sup>; Gavashelishvili, A.<sup>1</sup>; Gomez, C.<sup>1</sup>; Hillström, L.<sup>1</sup>; Janječić, M.<sup>1</sup>; Ježek, M.<sup>1</sup>; Keuling, O.<sup>1</sup>; Lefranc, H.<sup>1</sup>; Licoppe, A.<sup>1</sup>; Liefting, Y.<sup>1</sup>; Martinez-Carrasco, C.<sup>1</sup>; Olano, I.<sup>1</sup>; Palencia, P.<sup>1</sup>; Platovšek, Z.<sup>1</sup>, Plis, K.<sup>1</sup>; Podgórski, T.<sup>1</sup>; Pokorny, B.<sup>1</sup>; Rosell, C.<sup>1</sup>; Rowcliffe, M.<sup>1</sup>; Santos, J.<sup>1</sup>; Smith, G.<sup>1</sup>; Sola de la Torre, J.<sup>1</sup>; Soriguer, R.<sup>1</sup>; Stoyanov, S.<sup>1</sup>; Šprem, N.<sup>1</sup>; Zanet, S.<sup>1</sup>; Vicente Baños, J.<sup>1</sup>; Scandura, M.<sup>1</sup>

<sup>1</sup> Enetwild Consortium (affiliations are available on the request)

\* JoseAntonio.Blanco@uclm.es

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The European Observatory of Wildlife (EOW), within the ENETWILD project (www.enetwild.com, funded by the European Food Safety Authority), focuses on improving European wildlife monitoring by developing international data collection standards, supporting density estimation guidance, and fostering open, collaborative data networks. To achieve these objectives, EOW implemented a protocol using the random encounter model (REM) based on camera trap (CT) data for estimating mammalian density. This protocol integrates photogrammetry, allowing a three-dimensional reconstruction of camera scenes for calculating parameters essential to REM, including day range and detection values. The workflow, optimized on the Agouti platform, streamlines image processing and parameter estimation. Since 2021, ENETWILD stakeholders have employed the EOW protocol to assess densities of species like wild boar, roe deer, and red fox across Europe. By 2023, 30 institutions surveyed 44 sites in 22 countries, accumulating 79,092 CT activity days from 1,722 CT deployments. Density estimates show interannual stability with refined precision across several monitored populations. In 2024, network expansion included 40 institutions across 64 sites, prioritizing African swine fever (ASF)-impacted areas. 33 sites now fall within ASF-present countries, and 28 lie near the ASF frontline, highlighting regions where wild boar and domestic pigs coexist and where ASF risk is high. 20 sites (31%) contain an interface wild boar – domestic pigs, while 25 sites (39%) include wetland habitats, which are of possible epidemiological interest for avian influenza both within bird populations themselves and in relation to possible spill-over events to mammals (wild and domestic).

Ongoing networking efforts link EOW with other wildlife monitoring and disease-prevention initiatives, enhancing collaboration. The expansion of EOW sites aims to improve continental data representativeness, especially in regions where emerging diseases like ASF and avian influenza pose significant risks. The refined protocol, with augmented photogrammetry and Agouti functions, has enhanced data standardization and minimized user-related error. The EOW provides a perfect scenario to test improved approaches based on CTs but also novel technologies such as eDNA. EOW data supports continental-scale risk analysis, providing harmonized density estimates essential for disease modelling and aims to contribute to future schemes of biodiversity monitoring in Europe, such as the proposed EU Biodiversity Observation Coordination Centre (EBOCC). Consistent multi-year data collection across diverse ecological conditions will ensure comprehensive trend data for effective risk assessment in wildlife management.



### A new way to test live trapping: actual efficiency and animal welfare under the magnifier

Pontiggia, Pietro<sup>1,2,3,4\*</sup>; Focardi, Stefano<sup>2</sup>; Monaco, Andrea<sup>3</sup>; Franzetti, Barbara<sup>3</sup>; DeNicola, Vickie<sup>4</sup>

<sup>1</sup> University of Tor Vergata, Rome, Italy

<sup>2</sup> CNR-ISC – Institute for Complex Systems, Florence, Italy

<sup>3</sup> Italian Institute for Environmental Protection and Research (ISPRA), Rome, Italy

<sup>4</sup> Field Engine Wildlife Research and Management LLC, Moodus, USA

\* pietro.pontiggia@students.uniroma2.eu

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Wild boar (*Sus scrofa*) is arguably one of the most problematic species globally. The growth in numbers, coupled with the impact on natural ecosystems, agriculture and human activities, has prompted the implementation of intensive population control measures. Reducing wild boar numbers has become even more pressing as African swine fever spreads. This has resulted in an increased demand for intervention strategies to effectively address population reduction while ensuring efficiency and alignment with evolving animal welfare concerns. Therefore, we initiated a study which aims to evaluate and compare efficiency and animal welfare in three different trapping systems: modular trap, stationary trap, and net trap. The study has been carried out in the Presidential Estate of Castelporziano (Rome, Italy), a 6,000 ha large fenced area characterized by a Mediterranean environment and large biodiversity. The experimental design entailed the partitioning of the area into 3 subzones, which served as replications of the experiment. In each subzone, 4 units of each trap model were randomly deployed, for a total of 36 trapping sites throughout the 3 replications. A total of 50 successful trapping events were achieved between August and September 2024, resulting in a total capture of 154 wild boar (recaptures included). Following their capture, wild boar were marked, weighed, checked for the presence of injuries, and then released.

We used 3 camera traps to monitor each trap and determine the number of wild boar attracted, the composition of groups, and animal behaviour. Subsequently, the efficiency of each trap was determined by calculating the ratio of wild boar captured to the total number of wild boar present in the area. This ratio represents the actual trap's ability to successfully capture all animals that visited it during the conditioning phase. To investigate the variations in animal welfare among the 3 trap types, we conducted a comparative analysis of the impact of the trapping event on wild boar. We documented and assessed injuries and classified them into 11 classes according to severity.

The net trap exhibited a higher relative efficiency (0.90) than for the stationary trap (0.45) and modular trap (0.35), which is likely attributable to the distinctive operational characteristics and configuration of this trapping system. The incidence of damage was recorded in 41.3% of the animals trapped in stationary traps, 78.3% in the modular traps, and 0.0% in the net traps, respectively. The preliminary results indicate that the use of net traps represents an efficient and humane approach to the management of wild boar populations. Nevertheless, further data is required to reinforce this hypothesis and establish a functional protocol to enhance the efficacy of live trapping.



### Vanishing beasts: the conservation of African rhinoceros

Russo, Isa-Rita<sup>1\*</sup>

<sup>1</sup> Cardiff University, School of Biosciences, Cardiff, Wales, UK

\* russoim@cardiff.ac.auk

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Molecular tools are used for the assessment and monitoring of biodiversity which aid in conservation decisions and management. Recent developments in molecular methods have allowed researchers to analyse species at a genetic and/or genomic level. The level of analysis will depend on the available resources, funding, training, and access to technologies. In some countries/institutions, traditional genetic data such as microsatellites are still utilised in conservation decisions and in others advanced technologies that produce genome data for species are incorporated into management. Genetic/genomic information can answer a variety of research questions at either a higher evolutionary level or at phylogeographic, phylogenetic or population level. In this contribution, black rhinoceros is used as a case study to illustrate how genetic and genomic information can complement each other in conservation management. Genetic (microsatellites and mitochondrial DNA) data for black rhinoceros showed erosion of genetic diversity across the distributional range of the species and several evolutionarily significant units (ESUs) have been identified for the species. Data also showed that genome level analysis often identified finer scale patterns that were not observed using traditional markers such as mitochondrial DNA or microsatellite data, but the overall patterns of diversity were supported by all marker types. In addition, genomic data provided further information regarding inbreeding and genetic load. These genetic/genomic results should be implemented in future conservation actions for the species.



## Conservation management of an endangered endemic freshwater fish, *Sandelia bainsii*

Carr, Alexander<sup>1\*</sup>; Scheepers, Martinus<sup>2</sup>; Chakona, Albert<sup>2</sup>; Russo, Isa-Rita<sup>1</sup>

<sup>1</sup> Cardiff University, School of Biosciences, Cardiff, Wales, UK

<sup>2</sup> South African Institute for Aquatic Biodiversity, Makhanda, South Africa

\* carra2@cardiff.ac.auk

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Named after its province of origin in South Africa, the Eastern Cape Rocky (*Sandelia bainsii*) is an endangered freshwater apex predator. The introduction of invasive species such as bass and trout (*Micropterus* spp., *Salmo trutta* and *Oncorhynchus mykiss*) to river systems and dams for recreational and personal use has led to increased competition and predation. In addition, the construction of the Kat River Dam and the presence of many smaller weirs has caused large scale habitat fragmentation across the species' distribution. These factors are expected to have an adverse effect on gene flow between remnant populations that will affect the long-term evolutionary potential or persistence of the species. A mitogenomic study identified three allopatrically distributed lineages across the Eastern Cape Province, however further research into the genetic structure and diversity of the species is critically needed to inform targeted conservation management of vulnerable populations. Presented here is the first assessment of the species using novel microsatellite markers to identify current patterns of genetic diversity. Furthermore, we used Approximate Bayesian Computation (ABC) methods to determine historical demographic changes and infer the resilience or resistance of the species to future genetic pressures.



### Last of its kind: Danubian lacustrine brown trout (Salmo trutta)

Renko, Gašper<sup>1\*</sup>; Bravničar, Jernej<sup>1</sup>; Sušnik Bajec, Simona<sup>1</sup>; Palandačić, Anja<sup>2</sup>; Snoj, Aleš<sup>1</sup>

<sup>1</sup> University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia

<sup>2</sup> Vienna Museum of Natural History, First Zoological Department, Vienna, Austria

\* gasper.renko@bf.uni-lj.si

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Brown trout (*Salmo trutta*), native to Europe, North Africa and parts of Asia, has been introduced to various regions worldwide due to human-mediated translocations. This has resulted in the establishment of populations across all continents, except Antarctica, demonstrating the species' extraordinary adaptability and phenotypic plasticity. This ability to adapt to diverse aquatic environments and colonize new habitats is mirrored in the plethora of life histories a brown trout can adopt. Within lakes, brown trout gradually develop distinct characteristics over generations, adapting to lacustrine conditions and forming what is known as a lacustrine ecotype. The Danubian phylogenetic lineage of brown trout, native to Europe's largest river system, is renowned for producing large lacustrine individuals that historically thrived in Alpine lakes and supported local food needs. Regrettably, unfavourable management practices, introductions of non-native strains, and anthropogenic habitat degradation over the past century have likely led to the extinction of these iconic lake dwellers in their pure form.

To investigate the genetic composition and origins of potentially remnant Danubian lacustrine brown trout, we focused on populations inhabiting lakes Bled and Bohinj in Slovenia. Using microsatellite and mitochondrial DNA analysis on 365 specimens, we evaluated their genetic diversity and structure, comparing them to potential source populations. Additionally, low-coverage whole-genome sequencing was conducted to pinpoint genomic regions differentiating lacustrine and riverine populations and revealing signs of adaptation. Our findings revealed distinct genetic profiles between the two lake populations. Bohinj trout closely aligned with the native Danubian lineage, making it the last known population of non-hybridized Danubian lacustrine brown trout. In contrast, Bled trout displayed a hybrid composition with extensive influence from introduced Atlantic strains. The origin of the alien trouts was traced to Denmark and the most likely scenario for the formation of the hybrid swarm is discussed. These results highlight the complex genetic consequences of human-mediated introductions and emphasize the challenges in conserving natural populations amidst such pressures. It is imperative to implement urgent, ecotype-specific conservation measures to safeguard the native genetic pool of the Bohinj trout. Strategies should prioritize preserving the genetic integrity of this locally adapted population by restricting non-native introductions, mitigating habitat disturbances, and recognizing ecotypes as distinct conservation units. This approach is essential to prevent further genetic dilution and ensure the long-term survival of these unique populations, which hold significant ecological and cultural value.



# Status of rock partridge (*Alectoris graeca*) on the east coast of the Adriatic Sea

Rezić, Andrea<sup>1\*</sup>; Bužan, Elena<sup>2,3</sup>; Gančević, Pavao<sup>4</sup>; Fabijanić, Nera<sup>5</sup>; Pobega, Urška<sup>2</sup>; Šprem, Nikica<sup>1</sup>

- <sup>1</sup> University of Zagreb, Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Zagreb, Croatia
- <sup>2</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia
- <sup>3</sup> Faculty of Environmental Protection, Velenje, Slovenia
- <sup>4</sup> University of Split, Undergraduate Study of Mediterranean Agriculture, Split, Croatia
- <sup>5</sup> Croatian Hunting Federation, Zagreb, Croatia
- \* arezic@agr.hr

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The rock partridge (*Alectoris graeca*) is a mountain bird endemic to Europe. It is found in the Alps, the central and southern Apennines, Sicily in Italy, the Balkans, Albania, and Greece. In this distribution area, it mainly inhabits rocky areas and prefers dry, open habitats with sparse vegetation. Since 1950, rock partridge populations have declined across its range, with an estimated decline of almost 30% between 2011 and 2021, resulting in the species being classified as Near Threatened (NT) in both Europe and the EU28. The species was once widespread along the eastern coast of the Adriatic, but has disappeared from many of these areas due to various threats, including the abandonment of traditional farming practices in mountainous areas and habitat loss due to intensive agriculture. In addition, there is the overuse of pesticides, persistent drought, increasing predator populations, overhunting, and poaching. In some regions, such as the Alps, the Italian Apennines and Greece, these threats have led to the extinction of local populations.

To counteract this decline, captive-bred partridges, often chukars (*Alectoris chukar*) or hybrids between chukars and rock partridges, have been released on a large scale to replace native rock partridges. These releases have often occurred without a thorough assessment of hybridization risks, raising serious concerns about genetic pollution and the long-term conservation of rock partridge. Genetic admixture and introgression can disrupt local adaptations and potentially lead to population declines. Visual inspection alone cannot reliably determine the true origin or genetic differences of captive-bred partridges; therefore, genetic analysis provides a more accurate method to obtain this crucial information. Therefore, we studied the genetic makeup of rock partridges at 32 locations along the eastern Adriatic coast using 20 microsatellite markers to analyse genetic variation and population structure. This data can also serve as a basis for future genetic monitoring and conservation initiatives and help to ensure the long-term viability of the species.



## Genomic signature of bottleneck and founder effect in Alpine ibex populations in Slovenia

Bužan, Elena<sup>1,2\*</sup>; Pokorny, Boštjan<sup>2,3</sup>; Pobega, Urška<sup>1</sup>; Cidilko, Stefan<sup>1</sup>; Černe, Rok<sup>4</sup>; Nève Repe, Andreja<sup>4</sup>

- <sup>1</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia
- <sup>1</sup> Faculty of Environmental Protection, Velenje, Slovenia
- <sup>3</sup> Slovenian Forestry Institute, Ljubljana, Slovenia
- <sup>4</sup> Slovenia Forest Service, Ljubljana, Slovenia

\* elena.buzan@upr.si

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In Europe, Alpine ibex (*Capra ibex*) was on the brink of extinction in the 19<sup>th</sup> century, with the only surviving population in Gran Paradiso, Italy, which numbered around 100 individuals. Extreme historical bottleneck and founder effect have strongly shaped the genetic mak e-up of recent reintroduced populations across Alpine arc. The two only existing populations in Slovenia, i.e. in the Kamnik-Savinja Alps and the Julian Alps, were established by individuals originating from Gran Paradiso as well as from Swiss and Austrian populations, reintroduced before also from Gran Paradiso in the 19<sup>th</sup> and 20<sup>th</sup> centuries. Past analysis of mitochondrial and immunogenetic diversity from recent samples revealed limited haplotype variation in Alpine ibex from Slovenia, with only one predominant mitochondrial haplotype present in both the Julian Alps and the Kamnik-Savinja Alps. The MHC genetic diversity in the same populations showed presence of only one functional allele (Caib-DRB\*01).

The aim of this study was to investigate the signatures of inbreeding depression associated with founder events and population isolation in both populations using whole-genome sequencing at a 15-fold depth. Additionally, we included publicly available data on Alpine ibex from the NCBI database. Our analysis encompassed approximately 1 million unlinked nuclear SNPs as well as independent analyses of whole mtDNA and the Y chromosome. We demonstrated that, despite the rapid recovery of Slovenian populations and their increase in abundance during the 1960s, Alpine ibex exhibit persistent genomic signatures of historical bottlenecks, reintroduction events, and recent population declines. This is reflected in a low level of genome-wide genetic diversity compared to other established populations across the Alps. Our results highlight the urgent need to follow the results of the recent scientific research confirming that Alpine ibex is native to Slovenia; based on this, conservation actions aimed at improving genetic diversity of the species should be implemented as soon as possible. This is essential for increasing population viability and resistance to diseases.



### Genomic insights into hybridization and genetic introgression in European wildcats from the northern Dinaric Mountains and Pannonian Basin

Gerič, Urška<sup>1</sup>; Sollitto, Marco<sup>1</sup>; Šprem, Nikica<sup>2</sup>; Potočnik, Hubert<sup>3</sup>; Šnjegota, Dragana<sup>4</sup>; Ćirović, Duško<sup>5</sup>; Branković, Luka<sup>1</sup>; Bužan, Elena<sup>1,6\*</sup>

- <sup>1</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia
- <sup>2</sup> University of Zagreb, Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Zagreb, Croatia
- <sup>3</sup> University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
- <sup>4</sup> University of Banja Luka, Faculty of Natural Sciences and Mathematics, Banja Luka, Bosnia and Herzegovina
- <sup>5</sup> University of Belgrade, Faculty of Biology, Belgrade, Serbia
- <sup>6</sup> Faculty of Environmental Protection, Velenje, Slovenia
- \* elena.buzan@famnit.upr.si

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European wildcat (Felis silvestris silvestris) is facing severe threats due to recent habitat loss, fragmentation, and ongoing hybridization with domestic cat (Felis catus). The ancestor of today's domestic cat originated from African wildcat (Felis lybica) in the Near East and Egypt approximately 10,000 years ago. Historical and recent hybridization with domestic cats poses a significant challenge to the conservation of the genetic integrity of wildcat populations across Europe. The present study investigates the genomic structure of European wildcat populations in the northern Dinaric Mountains and the Pannonian Basin, focusing on the presence and extent of recent hybridization in Slovenia, Bosnia and Herzegovina, Croatia, and Serbia. We employed whole-genome sequencing at 10-fold depth for 40 wildcat samples, complemented by publicly available data on the Felis genus, to analyse approximately 1 million unlinked nuclear single-nucleotide polymorphisms (SNPs) and complete mitochondrial genomes. Two combined approaches were employed for the analysis of mitochondrial genome variation. The first approach involves assembling and annotating coding sequences, respectively with NOVOPlasty and MitoZ, followed by multiple alignment using MAFFT and phylogenetic tree construction with BEAST2. The second approach entailed analysing mitochondrial SNPs to construct a hierarchical clustering tree and perform MDS plot. These analyses confirmed the presence of genetic introgression in wildcats from Serbia, specifically around the Beograd region, where a high population of stray cats poses a significant risk of hybridization. By leveraging advanced genomic tools, this study provides valuable insights to improve conservation strategies aimed at preserving the genetic diversity and integrity of European wildcat populations.



# Developing a new panel of genomic loci for mammalian taxonomic identification: MIPs (Multi-locus Intron Polymorphisms)

Marinangeli, Lara<sup>1,2\*</sup>; Boscari, Elisa<sup>3</sup>; Scapolatiello, Annalisa<sup>3</sup>; Crestanello, Barbara<sup>1</sup>; Galla, Giulio<sup>1</sup>; Ferloni, Maria<sup>4</sup>; Piálek, Jaroslav<sup>5</sup>; Nonnis-Marzano, Francesco<sup>2</sup>; Congiu, Leonardo<sup>3,6,7</sup>; Hauffe, Heidi C.<sup>1,7</sup>

- <sup>1</sup> Fondazione Edmund Mach, Conservation Genomics Research Unit, Research and Innovation Centre, San Michele all'Adige, Italy
- <sup>2</sup> University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Parma, Italy
- <sup>3</sup> University of Padova, Department of Biology, Padova, Italy
- <sup>4</sup> Province of Sondrio, Sondrio, Italy
- <sup>5</sup> Institute of Vertebrate Biology, Czech Academy of Sciences, External Research Facility Studenec, Brno, Czech Republic
- <sup>6</sup> Consorzio Nazionale Inter-universitario per le Scienze del Mare (CoNISMa), Roma, Italy
- <sup>7</sup> National Biodiversity Future Center (NBFC), Palermo, Italy
- \* lara.marinangeli@unipr.it

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Rapid species identification is a fundamental tool for wildlife management and conservation plans. Many molecular methods have been proposed to solve taxonomic issues, such microsatellites (STR), or SNP (Single Nucleotide Polymorphisms) sets generated from RAD-seq and GBS (Genotyping-by-Sequencing). However, these markers tend to be species specific (STR) or cannot be efficiently applied to samples with scarce DNA quality/quantity (RAD-seq and GBS). Here we developed a new panel of Multi-locus Intron Polymorphisms ('MIPs') for mammalian genomic surveys, species and individual identification, and monitoring of interspecific hybridization. Specific subsets for rodents, lagomorphs, canids and their hybrids were designed. To generate a panel maximizing levels of cross-amplification, 121 available genomes from across Mammalia were aligned to identify the most conserved intronic regions; 192 introns randomly distributed across the genomes were then selected for the panel. Primer pairs suitable for multiplex PCR amplifications followed by Illumina amplicon sequencing were designed on conserved exonic flanking regions. The MIPs panel was tested on 71 laboratory mouse tissue samples to assess its potential to identify two parental lineages (Mus musculus musculus and M. m. domesticus) and different hybrid classes obtained from controlled laboratory crosses. The panel was then tested on invasive samples from several pairs of species known to hybridize under natural conditions: tissue samples from 250 mountain hares (Lepus timidus) and 70 brown hares (L. europaeus) bagged in the Province of Sondrio (Italy), from three hunting areas with different intensities of L. europaeus restocking, two altitudinal ranges (below and above 2000 m a.s.l.) and two collection periods (2001–2008 and 2016–2023) as proxies for climate change scenarios. Further tests were also conducted on other mammalian species belonging to the families Bovidae, Canidae, Felidae, and Suidae. Amplicon sequencing data were analysed with dedicated bioinformatic pipelines to assess inter- and intraspecific genetic diversity and level of hybridization of the populations under study. We discuss the usefulness of MIPs in addressing taxonomic problems and defining hybridization classes between different mammalian species.



# Craniometric characters of European badger (*Meles meles*) from the west Pannonian mountain

Krapinec, Krešimir<sup>1\*</sup>; Mijić, Marija<sup>2</sup>; Vucelja, Marko<sup>1</sup>; Tomljanović, Kristijan<sup>1</sup>; Konjević, Dean<sup>3</sup>; Bujanić, Miljenko<sup>3</sup>; Bjedov, Linda<sup>1</sup>

- <sup>1</sup> University of Zagreb, Faculty of Forestry and Wood Technology, Institute of Forest Protection and Wildlife Management, Zagreb, Croatia
- <sup>2</sup> Agriculture and Forestry School, Vinkovci, Croatia
- <sup>3</sup> University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia

\* kkrapinec@sumfak.unizg.hr

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Badgers are members of superfamily Musteloidea (Carnivora, Caniformia) with still controversial systematic relationships. Moreover, based on craniometric analysis from the beginning of the 21<sup>st</sup> century, four separate species were suggested within the genus of Eurasian badgers (*Meles* spp.). Despite the fact that European badger (*Meles meles*) was divided into two subspecies, this suggestion is still not generally accepted. However, because of the relatively wide distribution, some scull variabilities are possible due to more or less pronounced characteristics of sexual dimorphism.

The craniometric analysis was made on 62 skulls of European badgers, collected from Medvednica mountain (8450 ha) within the hunting years 2016/2017–2024/2025. Specimens were separated according to sex (male, female) and age (0 to 5 years, based on canines' cementum annuli). We measured 17 craniometric characteristics. Comparison was made between sex and age categories within Medvednica and among some other European populations (Carpathian, Fennoscandia). Badgers from Medvednica show relatively low level of sexual dimorphism, significant in only 5 craniometric characters – length of lower canines, basal length, condylobasal length, mandibular tooth-row length, and length of the first mandibular molar (M<sub>1</sub>). In general, badgers from Medvednica have larger skulls than badgers from Carpathian and Fennoscandian regions.



### Control of exotic disease outbreak in wildlife: bovine tuberculosis

#### Smith, Graham<sup>1\*</sup>

<sup>1</sup> National Wildlife Management Centre, APHA, Sand Hutton, York, UK

\* graham.smith@apha.gov.uk

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Control of exotic disease outbreaks in wildlife is made more difficult for two-species diseases that are chronic in nature. Bovine tuberculosis (TB) is such a disease when it has been transported by cattle movement into previously disease-free areas. I will describe several events where disease has been introduced through cattle movement and the adaptive management approach taken after discovering this disease has been transmitted into badgers (Meles meles), which are capable hosts. In the first case, identified in badgers in 2017 with the origin of infection being cattle imported from Ireland, the area for control had to be defined by surveying for badger main setts, identifying the probable infected areas by defining a minimum infected area (MIA) and adding a buffer onto this to account for incomplete information and spatial spread within badgers. The management approach in terms of culling or vaccination then had to be defined. Culled animals were examined post mortem and whole genome sequencing was used to examine the relationship between cattle and badgers, and the control strategy revised. This was repeated each year with the management moving from culling to vaccination, and now moving toward disease freedom. This will be the first eradication of bovine TB from the badger/cattle system in the UK. I will also report on four other outbreaks in the otherwise disease-free low risk area, and how these are progressing. This generic approach of defining an MIA and buffer has been used in other outbreak scenarios, and I will present the six necessary steps to perform adaptive management for other focal disease outbreaks.



# Tick-borne transmission of African swine fever virus in wild pigs: insights from Europe for preparedness in the USA

Wisely, Samantha M.<sup>1\*</sup>; Botero-Cañola, Sebastian<sup>1</sup>; Canino, Nicholas<sup>1</sup>; Torhorst, Carson<sup>1</sup>; Wilson, Kristen N.<sup>1</sup>, Davis, Angela<sup>2</sup>; O'Hara, Kathleen<sup>2</sup>

<sup>1</sup> University of Florida, Department of Wildlife Ecology and Conservation, Gainesville, USA

<sup>2</sup> United States Department of Agriculture, Center for Epidemiology and Animal Health, Fort Collins, USA

\* wisely@ufl.edu

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With its spread to the Caribbean basin, African swine fever virus has become a global panzootic of both wild and domestic suids. While the majority of transmission occurs from pig to pig or from fomite to pig, vector-borne transmission via Argasid soft ticks occurs in a sylvatic transmission cycle in Africa. In the Iberian Peninsula, soft ticks are associated with pig farms, and have been implicated in outbreaks of African swine fever. For example, the relevance of soft ticks in transmission was demonstrated in Spain in free ranging pigs living in old facilities (stone-made, extensive farming), but not for wild boar. Using lessons learned from the European experience with African swine fever and its tick vector, we created a species distribution map for *Ornithodoros turicata*, a soft tick in Florida, USA, and estimated contact rates between soft ticks and invasive wild pigs in order to develop a risk model for vector-borne transmission of African swine fever virus. Florida is considered a region at high risk for the importation of African swine fever virus, both because of its proximity to the Caribbean basin, but also because it has >1 million invasive wild pigs.

To create data for the model, we conducted stratified random surveillance of soft ticks in animal burrows throughout Florida. Using soft ticks collected from the sampling effort, we estimated contact rates between wild pigs and soft ticks by conducting molecular-based bloodmeal analysis. We surveyed 591 burrows at 113 sites across Florida, and collected ticks by vacuuming substrate from animal burrows and then sifting the substrate for ticks. 54% (61 of 113) of the sites had burrows that were occupied by *Ornithodoros turicata*. Of the 61 occupied sites, 10 sites had ticks with pig blood in their abdomen. Three additional sites had evidence of recent pig activity around the burrows. Taken together, 21% of sites had soft ticks that had either recently parasitized a wild pig, or a wild pig had come into the proximity of an infested burrow. These results strongly suggest that should the African swine fever virus establish itself in invasive wild pigs in Florida, there would be a high likelihood that it would also become endemic in soft ticks. Outbreak response plans should include a soft tick surveillance protocol, and based on a specific response plan African swine fever virus should be detected in *Ornithodoros turicata* in Florida.



# Golden jackal (*Canis aureus*) in the western Balkan epidemics of *Echinococcus multilocularis*

Csivincsik, Ágnes<sup>1\*</sup>; Moloi, Sibusiso<sup>1</sup>; Halász, Tibor<sup>1,2</sup>; Nagy, Gábor<sup>1</sup>

<sup>1</sup> Hungarian University of Agriculture and Life Sciences, Department of Animal Physiology and Animal Health, Kaposvár, Hungary

<sup>2</sup> SEFAG Forestry and Timber Industry Company, Department of Wildlife Management, Kaposvár, Hungary

\* csivincsik.agnes@uni-mate.hu

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*Echinococcus multilocularis* is a zoonotic tapeworm being responsible for the human alveolar echinococcosis (HAE), a chronic disease, which causes death of the patient in the lack of appropriate treatment. Wild Canidae play the role of definitive hosts in the life cycle of the parasite. Humans acquire infection accidentally and show liver lesions similar to those of the natural intermediate rodent hosts. The potential role of the golden jackal in the maintenance and spread of the parasite was initiated to investigate during the 2010s. Our research group joined this research in 2016. Since then, we have investigated more aspects of the host-parasite interactions between golden jackals and *E. multilocularis*. We compared the prevalence and mean intensity values of red fox and golden jackal, the egg production ability of the two hosts. We also evaluated environmental factors, which can contribute to the maintenance of *E. multilocularis* forming microfoci of the infection. We analysed the interdependence of human and carnivore epidemiology of *E. multilocularis*. Between 2016 and 2024, we investigated 435 golden jackals compared to red foxes (n=291).

We confirmed that prevalence and mean intensity were similar in the two species. Moreover, within some hotspots, the jackal population proved to be at a higher risk of infection. Our study on egg production confirmed the difference between the two hosts, which might cause multiplied risk by their coexistence. Among the environmental factors, wetlands were found to carry higher risk for maintenance of *E. multilocularis*, while annual precipitation and the seasonality of precipitation proved to have local effect on microfocus formation. During the years of investigation, we determined a high-risk cluster of *E. multilocularis* along the Drava River. By analysis of the correlations of HAE cases and the hotspots in wild carnivore, the human health risk due to presence and disease transmission by golden jackals proved to be suspicious. Our findings also support those of studies carried out in Croatia and Serbia, which call attention to the formation of a new *E. multilocularis* high risk area within the western Balkan region.



### Back to the future: pathogens as drivers of vole population cycles

Ecke, Frauke<sup>1,2\*</sup>; Evander, Magnus<sup>3</sup>; Khalil, Hussein<sup>2</sup>; Magnusson, Magnus<sup>2,4</sup>; Meheretu, Yonas<sup>2</sup>; Sjödin, Henrik<sup>5</sup>; Vanhatalo, Jarno<sup>1,6</sup>; Hörnfeldt, Birger<sup>2</sup>

- <sup>1</sup> University of Helsinki, Faculty of Biological and Environmental Sciences, Organismal and Evolutionary Biology Research Programme, Helsinki, Finland
- <sup>2</sup> Swedish University of Agricultural Sciences (SLU), Department of Wildlife, Fish, and Environmental Studies, Umeå, Sweden
- <sup>3</sup> Umeå University, Department of Clinical Microbiology, Umeå, Sweden
- <sup>4</sup> Swedish Forest Agency, Umeå, Sweden
- <sup>5</sup> Umeå University, Department of Epidemiology and Global Health, Umeå, Sweden
- <sup>6</sup> University of Helsinki, Faculty of Science, Department of Mathematics and Statistics, Helsinki, Finland
- \* frauke.ecke@helsinki.fi

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Already in the early era of quantitative population ecology, Charles Elton suggested diseases to regulate animal numbers. The refutation of this disease hypothesis in the 1950s has however caused pathogens getting low or almost no attention during the following period of "vole wars" where different schools of rodent researchers were competing to identify the likely misleading single factor driving cyclic dynamics of especially microtine rodents. Predation and food availability have been commonly proposed as the primary drivers. However, the predation hypothesis remains controversial, and interactions between multiple drivers are poorly understood. In addition, the emergence of new analytical techniques in recent decades has finally opened new cross-disciplinary research options which have increased our understanding of the distribution and ecology of rodent-borne pathogens fundamentally. The gained insights motivate us to revisit and revive the disease hypothesis. Indeed, there is mounting evidence that certain pathogens play an important role in the fitness of rodents. Here, we therefore revisit the disease hypothesis by studying and using a 42-year time series of field data, the role of food availability (i.e., seeds of Scots pine [*Pinus sylvestris*] and Norway spruce [*Picea alba*]), a common zoonotic pathogen (*Orthohantavirus puumalaense*), and predation by a specialist predator, for the population dynamics of bank voles (*Clethrionomys glareolus*).

We show that the interaction between pathogen infection and predation drives population cycles in bank voles. The vole, pathogen, and predator populations cycle synchronously. Dynamic modelling of the data revealed that vole cyclicity emerged only when both pathogen and predator were considered together, with the pathogen spreading efficiently in the vole population during winter, resulting in reduced reproduction in the following summer, and the predator likely increasing predation on infected voles. Our results demonstrate that pathogens play a critical role in vole population dynamics, suggesting that the disease hypothesis has been prematurely dismissed. The findings provide a basis for further exploration of multi-factor models in other cyclic species or for bank voles in other regions, examining the role of pathogens either alone or combined with other factors.



# Predictive relationships between rodent abundance and tick-borne disease patterns in central Europe

Tkadlec, Emil<sup>1\*</sup>; Kučerová, Monika<sup>1</sup>

<sup>1</sup> Palacký University, Department of Ecology and Environmental Sciences, Olomouc, Czech Republic

\* emil.tkadlec@upol.cz

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Small rodents are among the most significant reservoirs for many zoonotic pathogens, including those transmitted to humans by ticks. Annual incidences of tick-borne encephalitis and Lyme disease—the most severe tick-borne illnesses in central Europe—vary considerably from year to year. In Europe, the generalist tick Ixodes ricinus is the primary vector transmitting tick-borne pathogens to humans. This tick has three blood-feeding stages, with voles and mice acting as the main reservoir hosts for larval ticks. The transition from larva to nymph is a critical phase in pathogen transmission, as exposure to pathogens is most likely at this stage, directly affecting the density of infected nymphs. Vole population densities, in particular, change dramatically over time in 3-5-year cycles, influencing the likelihood that questing larvae will encounter a host and leading to significant variability in infection rates. Our previous research, based on a 17-year dataset, demonstrated that common vole (Microtus arvalis) abundance and climate variability can predict annual incidences of both diseases. Given the erratic nature of common vole population dynamics, we tested the robustness of these predictors with a longer data series of disease incidence from the Czech Republic. Our results confirmed that the predictive ability holds for tick-borne encephalitis but is weaker for Lyme disease. We propose a potential explanation for the stronger association between tick-borne encephalitis and the common vole.

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# Impact of broadleaved hedgerows on the distribution of rodentassociated pathogens and ticks in pine monocultures

Berard, Aloïs<sup>1\*</sup>; Galan, Maxime<sup>1</sup>; Pradel, Julien<sup>1</sup>; Loiseau, Anne<sup>1</sup>; Tatard, Caroline<sup>1</sup>; Castel, Guillaume<sup>1</sup>; Piry, Sylvain<sup>1</sup>; Berthier, Karine<sup>1,2</sup>; Moutailler, Sara<sup>3</sup>; Galon, Clemence<sup>3</sup>; Plat, Nattan<sup>4</sup>; Rivoal, Jean-Baptiste<sup>4</sup>; Jactel, Hervé<sup>4</sup>; Charbonnel, Nathalie<sup>1</sup>

- <sup>2</sup> INRAE, Pathologie végétale, Montfavet, France
- <sup>3</sup> ANSES INRAE EnvA UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, France
- <sup>4</sup> INRAE, BIOGECO, Cestas, France
- \* aloisberard@outlook.fr and alois.berard@inrae.fr

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Understanding the complex relationships between landscape and pathogens distribution in wildlife is crucial for evaluating and mitigating zoonotic risks. Ecological restoration has gained attention as a nature-based solution to bring back biodiversity in human-altered environments. However, the influence of restoration efforts on zoonotic risks remains poorly understood. In Western France, the intensively managed Landes Forest is dominated by maritime pine monoculture (>70%). The plantation of broadleaved hedgerows has been planned to restore forest biodiversity and improve forest resilience. However, the impact of these modifications on zoonotic risks has yet to be assessed.

To investigate whether hedgerows and landscape characteristics (i.e., connectivity and proportion of surrounding broadleaved vegetation) influence pathogen richness and prevalence in rodent populations, we conducted rodent and tick sampling in the Landes Forest during the spring and fall of 2023 and 2024. Our protocol included 53 trapping lines across 7 broadleaved forests, 24 broadleaved hedgerows, and 12 control trap lines along pine plot edges. Orthohantaviruses were detected using serological approaches. *Leptospira* spp. were analysed using a qPCR and the *lipL32* gene. Other pathogenic bacteria were detected with no *a priori* using a metabarcoding approach targeting the V4 region of the 16S-rRNA in the rodent spleen. *Bartonella* sp. identification was later refined using *gltA* and *rpoB* gene primers. Tick specimens were collected individually from rodents and via tick-flagging. Tick species and their pathogens' identification was achieved using a dedicated microfluidic chip.

Wood mouse (*Apodemus sylvaticus*) accounted for 95% of the 380 rodents trapped. Among the 311 wood mice analysed, we did not find any Orthohantavirus-seropositive individuals. We identified 12 putative pathogen bacteria (among which four *Bartonella* species, no *Leptospira* sp.) and three tick species. Notably, mice in area with fewer surrounding broadleaved trees showed a higher likelihood of infection with the tick-borne zoonotic pathogen *Neoehrlichia mikurensis*. Moreover, hedgerows exhibited a higher abundance of ticks than pine edges. These findings underscore the significant influence of landscape modifications on zoonotic hazards. Understanding the mechanisms underlying these relationships—such as shifts in host susceptibility, changes in host-pathogen encounter rates or altered vector distributions—is crucial for integrating effectively zoonotic risk management into ecological restoration practices.



<sup>&</sup>lt;sup>1</sup> INRAE, CBGP, Montpellier, France

# A machine learning model predicting the abundance of helminths of *Apodemus* mice in Vojvodina, Serbia

Tošić, Božana<sup>1\*</sup>; Gajić, Natalija<sup>2</sup>; Čabrilo, Borislav<sup>1</sup>; Bjelić-Čabrilo, Olivera<sup>1</sup>; Cvijanović, Dušanka<sup>1</sup>; Miljević, Milan<sup>3</sup>; Jakovetić, Dušan<sup>2</sup>

<sup>1</sup> University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia

<sup>2</sup> University of Novi Sad, Faculty of Sciences, Department of Mathematics and Informatics, Novi Sad, Serbia

<sup>3</sup> University of Belgrade, Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, Department of Genetic Research, Belgrade, Serbia

\* bozana.tosic@dbe.uns.ac.rs

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Research on the helminth fauna of small rodents was conducted in the period from 2019 to 2023 in the territory of Vojvodina Province, Serbia, in eight different localities. The rodent sample consisted of striped field mice (A. agrarius) (83), yellow-necked mice (A. flavicollis) (116) and wood mice (A. sylvaticus) (43). The mice were hosts to three helminth groups: nematodes, tapeworms, and digeneans. The aim of the study was to predict the abundance of helminths of selected species of the genera Aonchtotheca, Heligmosmoides, Syphacia and Trichuris, as well as species with zoonotic potential, i.e. Rodentolepis fraterna and Capillaria hepatica, based on various abiotic (Corine Land Use types, environmental variables, altitude, locality, region) and biotic (host species, sex, body mass, body length, spleen mass) factors. A random forest machine learning predictive model for factor importance evaluation was used to select and evaluate important features in predicting parasite abundance. Pvalues were estimated by using Monte Carlo analysis. The results showed that the prediction of the abundance of *C*. *hepatica* is influenced by the body condition index and spleen size of the host, and *R*. fraterna by the same two factors plus the mean monthly air temperature. The factors singled out as significant for species of the genus Heligmosomoides were numerous, including Corine Land Use types, all bioclimatic variables, and all biotic factors. Factors that significantly influenced the prediction of Syphacia and Trichuris species abundance were related to temperature, body condition index, and spleen mass of the host. As for Aonchotheca species, none of the factors were identified as significant. The obtained data are important from the aspect of using machine learning on these types of data and obtaining a better insight into parasite-host population dynamics, which is of particular importance when it comes to species that have zoonotic potential.

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# A camera trap protocol to determine micromammals abundance to support epidemiological studies

García-López, Virginia<sup>1</sup>; Vicente Baños, Joaquin<sup>1\*</sup>; Carro, Francisco<sup>2</sup>; Acevedo, Pelayo<sup>1</sup>, Ertürk, Alper<sup>1,3</sup>

<sup>1</sup> Universidad de Castilla-la-Mancha, Instituto de Investigación en Recursos Cinegéticos, Ciudad Real, Spain

<sup>2</sup> Research Biological Station (EBD), CSIC, Seville, Spain

<sup>3</sup> Kastamonu University, Kastamonu, Turkey

\* joaquin.vicente@uclm.es

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Management of wildlife communities and their ecosystems requires determining and monitoring changes in their specific composition and abundance. Micromammals are crucial species in ecosystems, playing a significant role as prey species and environmental engineers. However, they present complexity when developing monitoring programs. Density estimation of small mammals by capture-recapture generally involves handling them by rapid capture techniques, which sometimes results in the death of the animal, which is unethical. Therefore, we tested, for the first time, a timelapse based photo-trapping protocol (Space to Event model, STE) in an area of Doñana National Park (Southwest Spain) to determine the abundance of micromammals without the need to capture or handle them. The performed effort allowed determining the micromammal species richness present in the area and compared well against capture methodology. In terms of reliability of abundance estimation, while micromammal densities were within the range of estimations of minimum population size of micromammals determined in the area, its validation as a method to determine density of micromammals still require further efforts. We also evidenced the utility of this approach for monitoring other small-sized terrestrial wildlife, namely reptiles, indicating its potential for camera trap multispecies monitoring. In terms of practicability, field and data processing efforts are feasible and will only get better as new automatic identification tools are incorporated to this protocol. The aspects to improve the protocol include enhancing the optics of camera traps to visualize and identify species, as well as testing camera trap deployment distribution considering habitat availability in areas where, ideally, precise density of small terrestrial wildlife is reliably assessed by independent methods.



### Twenty years of rodenticide resistance monitoring in Belgium

Baert, Kristof<sup>1\*</sup>; Lathouwers, Michiel<sup>1</sup>

<sup>1</sup> Research Institute for Nature and Forest, Wildlife and Invasive Species Management, Brussels, Belgium \* kristof.baert@inbo.be

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Our research into anticoagulant rodenticides (AR) resistance in Flemish rat populations began in 2003 with blood clotting response (BCR) tests, in which small doses of ARs were administered to measure the prothrombin time and assess blood clotting effects. Over three years (2003-2005), we screened 691 rats from various locations for susceptibility to warfarin, bromadiolone, and difenacoum, identifying around 20% of the population as anticoagulant-resistant. Concurrent advances led to the discovery of the Vkorc1 gene and its role in rodenticide resistance. Genetic analysis of 119 rats revealed two prevalent mutations: Y139F and L120Q, each showing unique geographical distributions. Y139F was common in the western and eastern regions of Flanders, while L120Q appeared predominantly in the western central area, with notable absence of resistance in central Flanders. From 2006 to 2010, a second screening was conducted on 845 rats, of which 286 were at least warfarin-resistant. Depending on their BCR test result a total of 449 rats were genetically analysed, confirming the geographic spread of Y139F and L120Q and identifying for Flanders a rare Y139C mutation. Combined BCR and genetic findings highlighted Y139F's association with warfarin and bromadiolone resistance, while L120Q primarily caused warfarin resistance; both mutations showed very limited difenacoum resistance. In 2013, our screening methodology transitioned to a standardized genetic approach, focusing on SNP detection to replace BCR tests due to animal welfare and efficiency concerns. We aimed for a broad geographic spread by limiting samples to one per location due to spatial correlation, and targeted 1200 samples annually. This approach continued from 2013 to 2019, though 2017 and 2018 featured reduced sampling with 500 samples in central Flanders, where resistance prevalence was lower. By the end of this period, overall resistance had risen to 40%, influenced by gradual expansion of the resistant mutations' geographical range and increased local prevalence. Most recently, a 2024 screening revealed preliminary results indicating resistance levels nearing 50%. In some locations, rats were found to carry a combination of Y139F with either L120Q or Y139C mutations. Previous BCR tests in our lab on rats with the Y139F and L120Q combination indicated an increased degree of difenacoum resistance, suggesting potential challenges for rodent control in these areas. To evaluate whether practical implications of resistance have shifted over time, we recently conducted BCR tests on 67 rats from a historically high-resistance location (only with Y139F prevalence), specifically assessing for difenacoum resistance. Results showed that only 5 rats (7.5%) exhibited difenacoum resistance, a proportion that aligns with past findings and remains within acceptable limits for effective rodent control.

In conclusion, resistance has steadily increased over time, now reaching a concerning level of nearly 50%. Nonetheless, effective rodent management is achievable through targeted use of AR compounds based on the specific mutations present. Emphasis on preventive measures, mechanical trapping, and adherence to integrated pest management (IPM) practices will further support sustainable control efforts.



# Efficacy of brodifacoum in anticoagulant-resistant house mice (*Mus musculus*) of the hybrid L128S and Vkorc1<sup>spre</sup> introgression strain

Endepols, Stefan<sup>1</sup>; Richter, Dania<sup>2</sup>; Klemann, Nicole<sup>3</sup>

<sup>1</sup> Envu R&D, Monheim, Germany

<sup>2</sup> Labor Prof. Matuschka, Michendorf, Germany

<sup>3</sup> Consultant for rodent management and research, Warendorf, Germany

\* Stefan.endepols@envu.com

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The aim of this study was to assess the efficacy of 25 mg/kg brodifacoum in controlling anticoagulantresistant house mice (*Mus musculus*) of the hybrid L128S and Vkorc1<sup>spre</sup> introgression strain in feeding tests and in two practical treatments. Brodifacoum was proven effective in 4-days feeding tests with laboratory reared hybrid resistant mice. Two field studies were conducted with infestations comprising hybrid resistant mice, according to the ECHA guidance on the biocidal products regulation.

In the field study #1, the size of the initial infestation was estimated according to the mean consumption of 349 g/24h rolled oats during the pre-treatment census. After consumption of 1,113 g of bait, the control success was 100% and 99% according to the post-treatment feeding census and the tracking activity census, respectively. In the field study #2, the mean infestation size prior to the treatment was 59 g/24h. 324 g of bait was consumed during the treatment and resulted in a control success at 90% and 91% according to the feeding census and tracking census. The initial infestations comprised 16.7% (#1) and 100% (#2) of house mice of the hybrid L128S and Vkorc1<sup>spre</sup> strain. During the treatments, 27.8% and 60%, respectively, of succumbed house mice were hybrid resistant. The observed level of control was considered proof of efficacy of 25 mg/kg brodifacoum bait in controlling resistant house mice of the hybrid L128S and Vkorc1<sup>spre</sup> introgression strain.

The project was commissioned by RRAC.



### Brodifacoum – worrying trends in exposure in non-target wildlife

George, Sheila<sup>1\*</sup>; Campbell, Steve<sup>1</sup>

<sup>1</sup> Wildlife Management Unit, SASA, Edinburgh, UK

\* sheila.george@sasa.gov.scot

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Second generation anticoagulant rodenticides (SGARs), a key component of rodent management strategies, have been detected in a range of non-target wildlife species. In the UK, an industry-led stewardship scheme was introduced in 2016 in efforts to reduce this. The scheme developed compulsory best practice guidance on the use of anticoagulant rodenticides, introduced sales restrictions tied to a requirement for formal training and also monitors retailer compliance with the restrictions. Rule changes also permitted brodifacoum, difethialone and flocoumafen to be used outdoors 'in and around buildings', having previously been restricted to indoor use only. Brodifacoum is considered to be more potent than other dominant SGARs, so this rule change could have potential implications for non-target wildlife.

Here we present findings of recent research in Scotland, investigating the impact of stewardship on rate of exposure and concentration of SGARs in foxes and buzzards, with a particular focus on brodifacoum. We also briefly discuss trends in brodifacoum exposure in other species and provide context on rodenticide use from surveys of key sectors. No reduction in rate of exposure or concentration of SGARs was observed in study species. Exposure to brodifacoum doubled in foxes and almost tripled in buzzards post-stewardship. They were both also more likely to be exposed to multiple SGARs post-stewardship. Recent rodenticide usage surveys suggest that brodifacoum represents a greater proportion of total rodenticide use, in sectors for which data are available, has decreased in the same time period. In the absence of sales data, it is unclear whether the sectors surveyed are representative of total use, or if poor compliance could explain the increase in wildlife exposure to brodifacoum.

This work adds to a growing body of evidence suggesting that the UK industry-led stewardship scheme has not yet met its objective of reducing non-target wildlife exposure to rodenticides. The increase in the frequency of brodifacoum exposure in all species investigated is of particular concern. Continued monitoring of residues will be important as future rule changes are introduced. Further modifications to rodenticide usage under stewardship may also be necessary to reduce non-target exposure of wildlife to anticoagulant rodenticides.



## Environmental exposure assessment of anticoagulant rodenticides and $\alpha$ -chloralose in domestic cats (*Felis catus*) in Slovenia

Cerkvenik-Flajs, Vesna<sup>1\*</sup>; Schenke, Detlef<sup>2</sup>; Korenjak-Černe, Simona<sup>3</sup>; Perpar, Anton<sup>4</sup>; Jacob, Jens<sup>5</sup>; Schwonbeck, Susanne<sup>6</sup>; Kleine Bardenhorst, Sven<sup>7</sup>; Torsten, Hahn<sup>6</sup>; Cvetko, Marko<sup>1</sup>; Gombač, Mitja<sup>1</sup>

- <sup>1</sup> University of Ljubljana, Veterinary Faculty, Institute of Pathology, Wild Animals, Fish and Bees, Ljubljana, Slovenia
- <sup>2</sup> Julius Kühn Institute (JKI) Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany
- <sup>3</sup> University of Ljubljana, School of Economics and Business & Institute of Mathematics, Physics and Mechanics, Ljubljana, Slovenia
- <sup>4</sup> University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Ljubljana, Slovenia
- <sup>5</sup> Julius Kühn-Institute (JKI) Federal Research Centre for Cultivated Plants, Rodent Research, Münster, Germany
- <sup>6</sup> Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany
- <sup>7</sup> University of Münster, Institute of Epidemiology and Social Medicine, Clinical Epidemiology, Germany
- \* vesna.cerkvenik.flajs@vf.uni-lj.si

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Rodenticides as biocidal products are regulated by the Biocidal Products Regulation (EU) 528/2012 and 11 of the originally authorized 16 active substances are now in a renewal phase. Risk assessment including the assessment of secondary exposure is part of the substance evaluation. It is particularly important for anticoagulant rodenticides (ARs) that can be harmful to non-target wildlife and domestic animals. The residues of eight ARs and  $\alpha$ -chloralose were monitored in domestic cats (*Felis catus*) in Slovenia. From 2021 to 2022, carcasses of 99 animals originating from veterinary clinics, animal shelters and roadkill were collected. Rodenticides were extracted from liver tissue and measured by liquid chromatography-electrospray tandem mass spectrometry. Residues of at least one rodenticide were detected in 65% of samples. The ARs brodifacoum (54% of samples), bromadiolone (25%) and coumatetralyl (21%) were most common. We determined brodifacoum at concentrations of ≥800 ng/g in 3 of 99 cat liver (3%), and one of these (1%) contained residues of >1,800 ng/g, which seems sufficient to cause mortality.  $\alpha$ -chloralose was found in one sample (1.0%) at 561.7 ng/g. Two older animals living outdoors contained hepatic residues of four ARs. Exposure of cats to ARs is prevalent but seems lower than in predatory wildlife.

Individual characteristics of cats (sex, age, roaming in/outdoors) did not affect the occurrence or concentration of liver residues but environmental factors did. The number of rodenticides present was positively associated with human population density as well as rural land use and was negatively associated with farm density. A similar trend was evident for the presence of brodifacoum and bromadiolone. The concentration of brodifacoum was higher at low farm density.

The results highlight a potential environmental problem related to the ARs in a pet species – a group of animals rarely considered in this regard. As the study indicates an ecotoxicological risk for domestic cats in Slovenia, any kind of asymptomatic secondary poisoning related to rodenticides should be monitored in the future to identify the relevance of the issue apart from mortality.









### Rodent damage in sugarcane

Harpering, Philipp<sup>1</sup>; Kammerer, Christian<sup>2</sup>; Naranjo, Moramay<sup>3</sup>; Luzuriaga, Diego<sup>3</sup>; Ellington, Hance<sup>4</sup>; Shiels, Aaron<sup>5</sup>; Jacob, Jens<sup>1\*</sup>

- <sup>1</sup> Julius Kühn-Institute (JKI) Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Rodent Research, Münster, Germany
- <sup>2</sup> Hendry County Extension Service, LaBelle, Florida, USA
- <sup>3</sup> Florida Crystals Corporation, West Palm Beach, Florida, USA
- <sup>4</sup> University of Florida, Range Cattle Research and Education Center, Ona, Florida, USA
- <sup>5</sup> National Wildlife Research Center, Fort Collins, Colorado, USA
- \* jens.jacob@julius-kuehn.de

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Rat damage in sugarcane fields can lead to significant loss in biomass and sugar content and ultimately in reduced sugar production. Often, damage is patchy, probably as a result of competition, predation, resource availability, microhabitat effects, and spatial distribution of rodents.

We assessed the presence of rodents and the occurrence of rodent damage to sugarcane in a 200,000 ha sugarcane cropping area in southern Florida, USA. Rodent damage was estimated from 2017-2020 and 2024 by sampling stalks during the harvest period across various fields and farms. Damaged and undamaged stalks were counted and weighed to estimate the extent of the damage. The stalks were milled, and the sugar content of the juice was measured. In 2024, rodents were trapped using snap traps. Five rodent species were present in the fields: house rat (*Rattus rattus*), brown rat (*R. norvegicus*), house mouse (*Mus musculus*), marsh rice rat (*Oryzomys palustris*), and cotton rat (*Sigmodon hispidus*). The former was the most abundant rodent. Rodent damage varied temporally mainly driven by sugar content. It also varied spatially because rodents seemed to accumulate in plots harvested late. In addition, microhabitat features mattered. We present an overview of the extent of damage and losses inflicted by rodents, the temporal and spatial distribution of damage, and suggestions on how to mitigate losses in the future.



# Ecologically based rodent management in post-harvest: progress and success

Htwe, Nyo Me<sup>1\*</sup>; Stejskal, Vaclav<sup>2,3</sup>; Belmain, Steven R.<sup>4</sup>; Singleton, Grant R.<sup>4</sup>; Jacob, Jens<sup>1</sup>

<sup>1</sup> Julius Kühn Institute (JKI), Federal Research Institute for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Rodent Research, Münster, Germany

<sup>2</sup> Crop Research Institute, Prague, Czech Republic

<sup>3</sup> Czech University of Life Sciences, Prague, Czech Republic

<sup>4</sup> University of Greenwich, Natural Resources Institute, Kent, United Kingdom

\* nyo.me.htwe@julius-kuehn.de

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The high losses caused by rodents to cereal grains in storage have a disproportionately high impact in smallholder farming communities in low and medium income countries (LMICs). Many are subsistence farmers, and they can ill afford losses to rodents after investing money and labour into the production and harvest of their crop. Post-harvest rodent impacts in high income countries (HICs) are also apparent, often in terms of quality loss. We review the ecology of different rodent species and associated management systems during storage in LMICs as well as in HICs.

In LMICs, rodenticides and community trapping alone have been shown to reduce rodent populations, damage and contamination, however, populations quickly rebound if control is not maintained. The main rodent pest species attacking grain storage often live in both field and community areas. Ecologically based rodent management (EBRM) in post-harvest systems in Tanzania and Myanmar highlighted the importance of integrating on-farm and rural community rodent management. In HICs, warehouses on farms and food processing operations also face a number of specific rodent control related problems. This is particularly related to the lack of safe chemical alternatives to anticoagulant baits, associated with environmentally unfriendly impacts (secondary exposure of non-target animals), especially those linked to bait-based rodent control outdoors and barrier baiting around warehouses. Additionally, the reduction in the concentration of active ingredients in baits and the growing resistance to anticoagulants are significant concerns. This implies the necessity of developing more comprehensive, targeted integrated pest management (IPM) strategies that will rely less on chemical control and more on advanced methods such as electronic distance monitoring, mechanical trapping, and exclusion. Proper sanitation and exclusion of rodents integrated with regular monitoring for the presence of rodents followed by quick management action when rodent signs are above a threshold are key EBRM practices for managing post-harvest impacts of rodents.



# Can repellent plants reduce the abundance of the common vole (*Microtus arvalis*)?

Suchomel, Josef<sup>1\*</sup>; Šipoš, Jan<sup>1</sup>; Heroldová, Marta<sup>2</sup>

<sup>2</sup> Mendel University in Brno, Department of Forest Ecology, Brno, Czech Republic

\* suchomel@mendelu.cz

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Common vole (Microtus arvalis) is one of the most damaging rodents in agriculture. A number of methods are used to suppress its numbers and limit damage. One option is growing crops that are not suitable for voles; this might limit their numbers and distribution. Through long-term monitoring of common voles throughout the Czech Republic, their abundance in eleven crop types (annual, biennial and perennial crops) was evaluated through active burrow counts. Reference crops were selected from perennials and biennials and annual crops were selected as potential repellents. The perennial crops were clover with alfalfa, which serve as the primary habitat for voles. These crops are where voles are found in their highest densities, and from which they spread to the surrounding crops during periods of high abundance. The biennial winter rape was selected because it provides favourable conditions for voles to overwinter and multiply rapidly in the spring. Compared to perennial crops and winter rape, significantly lower numbers of voles were found in onions, poppy seeds, and maize. Additionally, fewer voles were also found in mustard compared to perennial crops. Onion and poppy were the only crops to show a significantly lower abundance of voles in comparison to wheat. The annual crops tested are unattractive to voles and due to the cultivation practices used, they do not even have enough weeds as food. As profitable crops they can be cultivated over sufficiently large areas to potentially prevent the spread of voles to surrounding crops, especially during outbreak years.

This study was supported by the Technology Agency of the Czech Republic, the project SS06020333: Suppression of common vole (*Microtus arvalis*) population densities using natural plant repellents.



<sup>&</sup>lt;sup>1</sup> Mendel University in Brno, Department of Zoology, Fisheries, Hydrobiology and Apiculture, Brno, Czech Republic

# Adaptability of the breeding phenology of birds of prey occupying nest boxes in the study areas for biological control of the common vole in agricultural environments in Castilla y León (Spain)

Báscones-Reina, Miriam<sup>1</sup>; Hernández-Garavís, Lorena<sup>1</sup>; Garcés-Toledano, José Fernando<sup>1</sup>; Cuéllar-Basterrechea, Carlos<sup>1</sup>; González-Simón, Paula<sup>1</sup>; Rodríguez-Moreno, Beatriz<sup>1</sup>; Blanca-Chana, Fernando<sup>1</sup>; Caminero-Saldaña, Constantino<sup>1</sup>

<sup>1</sup> Group for the Rehabilitation of Native Fauna and its Habitat (GREFA), Majadahonda, Spain \* miriam@grefa.org

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Adaptation during the breeding season is a key aspect of the ecology of raptors, particularly those, like barn owl (*Tyto alba*) and common kestrel (*Falco tinnunculus*), that rely on prey with cyclic populations, such as the common vole (*Microtus arvalis*). These rodents exhibit significant population fluctuations, which can directly impact the availability of food for specialized predators and, consequently, the timing and success of their breeding. Nest boxes have proven to be valuable tools for studying and monitoring raptor populations in agricultural environments, allowing us to investigate how these birds adapt their breeding patterns to changes in prey abundance. The peak energy demand for raptors that aims to increase their hunting rate coincides with their breeding season, from egg-laying to fledging. Given the potential of raptors as biological control agents for vole pests comparable to other phytosanitary measures, it is relevant to determine the duration and persistence of their predatory impact, as well as its temporal alignment with periods of peak pest pressure when the control is most needed.

This study explores the adaptive capacity of nest-box raptors during the breeding season, focusing on their response to changes in vole abundance. By analysing field data, we aim to determine whether these birds possess behavioural adaptations in their breeding phenology that optimize reproductive success under variable prey conditions. This research may contribute to our understanding of the efficacy of biological control as an integrated pest management strategy for vole pests, particularly in agricultural landscapes where predator-prey dynamics may be influenced by human activities.



# The barn owl and its predatory capacity against rodent pests: a case study on its role in biological control of the common vole

Hernández-Garavís, Lorena<sup>1\*</sup>; Báscones-Reina, Miriam<sup>1</sup>; González-Simón, Paula<sup>1</sup>; Garcés-Toledano, José Fernando<sup>1</sup>; Cuéllar-Basterrechea, Carlos<sup>1</sup>; Rodríguez-Moreno, Beatriz<sup>1</sup>; Blanca-Chana, Fernando<sup>1</sup>; Caminero-Saldaña, Constantino<sup>1</sup>

<sup>1</sup> Group for the Rehabilitation of Native Fauna and its Habitat (GREFA), Majadahonda, Spain

\* garavis@grefa.org

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The common vole (*Microtus arvalis*) is a pest that causes significant damage to agriculture in certain areas of the Spanish cereal steppe. To promote biological control, nest boxes for barn owls (*Tyto alba*) have been installed in affected areas. Using camera traps, a barn owl family was monitored for two years, one with high vole abundance and the other with low abundance. This study, although with a limited sample size, aims to illustrate the potential of the barn owl as a control agent during a common vole outbreak. The objectives were to quantify prey consumption by a barn owl family during its breeding cycle, to assess whether the hunting activity of this raptor varies between different reproductive stages (incubation, chick rearing) and between years with different vole abundance.

Preliminary results reveal a significant increase in vole consumption by barn owls during years of high vole abundance, highlighting their role as natural biological controllers. These findings quantify the valuable ecosystem service provided by barn owls and provide a basis for promoting their conservation and encouraging their use as a tool for integrated pest management at local and regional levels in areas with similar conflicts.



# Prey structure of three sympatric predators of small mammals: tawny owl, barn owl, and long-eared owl

Janžekovič, Franc<sup>1\*</sup>; Klenovšek, Tina<sup>1</sup>

<sup>1</sup> University of Maribor, Faculty of Natural Sciences and Mathematics, Maribor, Slovenia

\* franc.janzekovic@um.si

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Long-eared owl (*Asio otus*), tawny owl (*Strix aluco*), and the barn owl (*Tyto alba*) coexist in the lowland areas of north-eastern Slovenia. All three species are nocturnal predators of small mammals and, to a lesser extent, birds, reptiles, amphibians, and insects. Their habitat encompasses rural, suburban, and urban cultural landscapes. The long-eared owl predominantly hunts in open areas, particularly grasslands and agricultural fields, and specializes in a few species of voles and mice. Tawny owl mainly hunts in forest habitats and forest edges, preying on a diverse spectrum of woodland small mammals. Barn owl mostly capture their prey in urban and suburban environments, with a relatively balanced representation of small mammals. All three owl species employ a similar hunting strategy, combining sit-and-wait tactics with ambush hunting. Due to the overlap in three aspects of their ecology—their sympatry, similar prey selection, and comparable hunting behaviour—competition among these species is highly likely.

Our study focused on the taxonomic, functional, and phylogenetic diversity of the prey of the three owl species. We investigated whether the functional traits of prey differ depending on the predator species. We assumed that owls avoid competition by specializing in distinct prey groups. Additionally, we examined the phylogenetic composition of prey of each predator. Biodiversity was quantified with Hill numbers. We analysed 28 prey samples comprising 32 prey species and 10,049 individual specimens. In terms of species richness, the highest taxonomic diversity was observed in barn owl's diet, with a median of 16 species, followed by tawny owl's diet, with a median of 13 species per sample. Longeared owls exhibited significantly lower prey biodiversity, with a median of 9 species. In functional and phylogenetic diversity, tawny owl's prey showed the highest diversity, although it did not significantly differ from barn owl's prey. In contrast, the long-eared owl's diet was notably monotonous and significantly less diverse than the diets of the other two species. In terms of dominant prey species, which constituted over 75% of the total prey, long-eared owls diet included only three taxa, whereas the diets of both tawny and barn owls included seven taxa. Functionally, long-eared owls preyed on an average of only 1.1 taxa, compared to 1.5 for tawny owls and 1.4 for barn owls, respectively. Similarly, in phylogenetic diversity, long-eared owls prey averaged 1.1 taxa, while tawny owls and barn owls prey contained 1.6 and 1.3 taxa, respectively.

Our findings indicate significant differences in prey composition across all biodiversity dimensions. Long-eared owl is a highly specialized predator, focusing on a limited number of prey species in open habitats. The prey composition of tawny owl and barn owl is similar across all aspects of biodiversity, with differences primarily related to hunting habitats. Tawny owl predominantly preys on forestdwelling voles and mice, whereas barn owl targets species adapted to more urbanized environments.



# Seasonal movements of GPS-tracked Eleonora's falcons (*Falco eleonorae*) from La Galite Archipelago, Tunisia

Monti, Flavio<sup>1\*</sup>; Ouni, Ridha<sup>2</sup>; Tillo, Stephan<sup>3</sup>; Shaiek, Moez<sup>4</sup>; Dorai, Sahbi<sup>4</sup>; Zaghdoudi, Hassen<sup>5</sup>; Delaugerre, Michel-Jean<sup>6</sup>; Mayol, Joan<sup>7</sup>; Croizé, Nicolas<sup>7</sup>; Tankovic, Eva<sup>7</sup>; Chaumont, Jeanne<sup>7</sup>

- <sup>1</sup> Institute of Research on Terrestrial Ecosystems (IRET), National Research Council (CNR), Campus Ecotekne, Lecce, Italy
- <sup>2</sup> Independent Researcher, Sidi Thabet, Tunis, Tunisia
- <sup>3</sup> Independent Researcher, Rouet, France
- <sup>4</sup> Méditerranée Action Nature (MAN), Zarzouna, Tunisia
- <sup>5</sup> Agence de Protection et d'Aménagement du Littoral (APAL), Tunis, Tunisia
- <sup>6</sup> Independent Researcher, Bastia, Corsica, France
- <sup>7</sup> PIM Initiative for Small Mediterranean Islands, Marseille, France
- \* flavio.monti@cnr.it

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To effectively conserve migratory land bird populations during the full annual cycle, it is important to have in-depth knowledge of their spatiotemporal distribution throughout the year. Indeed, longdistance migratory populations may exhibit dissimilarities in the timing of migration, the routes they follow and convergence at ecological barriers. In this sense, tracking studies help to investigate individual-level decisions taken en route across seasonal journeys, providing key information to suggest management actions and conservation measures at the flyway scale. Here, we provide the first description of the seasonal movements of six Eleonora's falcons Falco eleonorae (four adults and two juveniles) tracked by GPS/GSM devices from Gallo islet, in the La Galite Archipelago (Tunisia). Birds were tagged at their breeding/natal colony in the second half of September, coinciding with the last phases of the chick-rearing period. Adults performed repeated foraging trips at sea, showing a preferred SW-NE orientation from the colony to the open sea, in order to intercept the flow of migrating passerines. Several trips to the coasts and inland areas of northern Tunisia were also recorded, until autumn migration. Except for an immature female that, after an explorative movement in Sardinia and Sicily, departed for migration on 11 October, the other three adults and two juveniles left the colony between end of October and beginning of November (27 Oct to 11 Nov). During autumn migration, the four adults followed a similar route across Libya, Chad and South Sudan, through the rainforest and towards Lake Victoria, to the east coast of Mozambique where they crossed the sea to reach their wintering grounds in northern Madagascar (end of November). The two juveniles, once left Tunisia, took a more western route across the Sahara Desert of Libya and Niger, up to Nigeria and Cameroon: from there they reoriented and converged towards Lake Victoria, and are still on the way at the time of writing the abstract. These findings represent the first data on the movement ecology of this species for North Africa, and are in line with international initiatives in other Mediterranean countries aimed at integrating research, policy and action for the conservation of these Afro-Palearctic migratory land birds at the flyway scale.



### An excursus on the role of wildlife carcasses

Jordan-Fragstein, Claudia C.1\*; Müller, Michael G.1

<sup>1</sup> TUD Dresden University of Technology, Chair of Forest Protection, Tharandt, Germany

\* claudia.jordan-fragstein@tu-dresden.de

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Wildlife carcasses serve as biodiversity hotspots for soil bacteria and fungi, as well as for bacteria and fungi that inhabit or grow on the carcass itself (the necrobiome). They also play a vital role in supporting scavenging wildlife, particularly vulture species, which rely on the availability of carrion. Due to aesthetic and veterinary regulations, however, we rarely or never encounter carcasses in the wild. The rapid removal of carcasses naturally impacts biodiversity by disrupting food chains and reducing habitat complexity.

Examples from a vulture project in Namibia, as well as carcass research in Germany using exposed large vertebrate carcasses, provide insights into the significance of carcasses for scavenging wildlife, habitat parameters in the immediate vicinity of carcasses, and the effects on bird of prey populations, such as vultures. These studies highlight the essential role carcasses play not only in sustaining scavenger populations but also in shaping local biodiversity and ecological balance. Thus, rapid carcass removal can have significant ecological consequences. Future research and management should take into account the crucial role of carcasses in ecosystems to preserve biodiversity and support the health of scavenger species and their habitats.



#### Den use in captivity: insights from two bears

Nikolova, Danita<sup>1</sup>; Spasova, Venislava<sup>1\*</sup>; Simeonovska-Nikolova, Daniela<sup>1</sup>; Dimitrov, Krastio<sup>1</sup>; Zareva-Simeonova, Katerina<sup>1</sup>; Todorov, Vladimir<sup>2</sup>; Hristov, Hristian<sup>3</sup>

<sup>1</sup> Sofia University "St. Kliment Ohridski", Department of Ecology and Environmental Protection, Sofia, Bulgaria

<sup>2</sup> Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

<sup>3</sup> Nature and Animal Conservation Centre, Dobrich, Bulgaria

\* venislavar@yahoo.com

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Brown bears in captivity could provide valuable insights into their den-related behaviour. We monitored two brown bears (*Ursus arctos*), a male and a female in the Nature and Animal Conservation Centre (Zoo Centre) in Dobrich City, Bulgaria, during the denning period. The bears lived together in an enclosure with two dens dug by the bears and yearly used. From November to March 2022 and 2023, camera traps were installed in front of each den to record the behaviour of the two individuals near the dens. The study revealed details about the den chronology, when each bear enters and exits as well as some individual differences between both individuals. A hierarchy regarding den use, with the female being dominant, was established. These findings enhance our understanding of den-related behaviour and may help to improve the welfare of the brown bear.



# The effects of sex, age, season and habitat on the diet of red fox (*Vulpes vulpes*) in Western and Central Lithuania

Špinkytė-Bačkaitienė, Renata<sup>1\*</sup>; Kurpeikis, Giedrius<sup>1</sup>; Kibiša, Artūras<sup>1</sup>

<sup>1</sup> Vytautas Magnus University, Faculty of Forestry and Ecology, Kaunas, Lithuania

\* renata.spinkyte-backaitiene@vdu.lt

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We investigated the dietary diversity of red fox (*Vulpes vulpes*) in different seasons depending on the individual's sex and age in Western and Central Lithuania. The objectives were: (a) to assess the dietary diversity depending on the sex and age of the individual; (b) to investigate the dietary diversity of foxes in different seasons; (c) to investigate the dietary diversity of foxes in different regions of Lithuania; (d) to assess the effect of health on the diet of foxes.

465 red fox carcasses were collected from October 2021 to December 2023, and their diet was investigated by stomach content analysis in two regions of Lithuania. Additionally, each fox was weighed, measured, sexed, aged, and examined for scabies infection. Food remains were detected in 379 stomachs (81.5%). The study showed that small rodents (*Microtus*) dominated in the foxes' diet (51.6% of stomachs). Other food remains were found less frequently: carrion (24.4%), birds (18.8%), fish (4.9%), invertebrates (45.0%), fruits, seeds and vegetables (39.4%), plant materials (55.0%), anthropogenic materials (1.7%), and others (1.3%). Characteristic differences in dietary diversity were found depending on sex and age. The food niche of adult male and juvenile foxes was more diverse than that of adult females. Based on our findings, red foxes can be recognised as omnivorous predators, feeding on readily available and abundant prey. 46 individuals were found infected with scabies, representing (9.9%), therefore the healthy individuals represented 90.1% of studied foxes. The diet was not affected by the scabies infection.



# A comparative analysis of activity patterns and temporal niche overlap between golden jackal (*Canis aureus*) and red fox (*Vulpes vulpes*): south Slovakia vs. northeast Italy

Comuzzi, Alice1\*; Guimaraes, Nuno2; Filacorda, Stefano1

<sup>1</sup> University of Udine, Department of Agri-Food, Environmental and Animal Sciences, Udine, Italy

<sup>2</sup> Technical University in Zvolen, Faculty of Forestry, Department of Applied Zoology and Wildlife Management, Zvolen, Slovakia

\* comuzzi.alice@spes.uniud.it

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Coexisting species often adopt strategies to minimize competition for shared resources. While various mechanisms can facilitate coexistence, usually subordinate species adjust their ecological niche to avoid direct encounters with dominant competitors. This study examined temporal niche partitioning between two mesocarnivores: golden jackal (*Canis aureus*) and red fox (*Vulpes vulpes*), two sympatric canids with a high potential competition due to their similar ecological requirements. To gain a significant understanding of the coexistence mechanisms between the target species we used data from two different camera-trap surveys conducted in October and November 2023 in two distinct areas: Friuli Venezia Giulia region (north-east Italy) (hereafter, FVG) and the protected landscape area of Cerová Vrchovina (hereafter, CV PLA) in Slovakia. We hypothesize that both mesocarnivores would exhibit a similar temporal use of habitat in CV PLA, while in FVG, there would be a greater degree of temporal niche separation. The study areas were divided into equal sampling units (SAs) using a 3.3 x 3.3 km grid with a total of 23 squares: 15 in CV PLA and 8 in FVG, within which there were 35 camera-trap stations: 19 in CV PLA and 16 in FVG. There were in total 869 trapping days in CV PLA (on average 46 days per SA) and 572 trapping days in FVG (on average 36 days per SA).

Results showed a higher RAI (relative abundance index) for red fox in CV PLA (33.13) compared to FVG (5.42), and conversely a higher RAI for golden jackal in FVG (6.12) compared to CV PLA (2.65). The naïve occupancy for red fox was 1.00 in CV PLA and 0.44 in FVG, while the one of golden jackal was 0.11 in CV PLA and 0.31 in FVG. Results also revealed a higher temporal overlap between the target species in CV PLA compared to FVG, suggesting greater avoidance of foxes towards jackals in the latter region. Several factors could explain these results: the higher detections of jackals in FVG compared to foxes, and the opposite pattern in CV PLA; the uniform distribution of the fox in all camera-trap locations in CV PLA; the lower naïve occupancy of golden jackal in CV PLA compared to FVG. Potentially other factors could be the higher human disturbance and agricultural activity in FVG versus more extensive forest cover in CV PLA, and the higher detections of potential prey for both species in CV PLA compared to FVG. The results of this study partially validated our predictions, indicating that the target species adopted distinct temporal patterns to coexist, likely due to ecological dissimilarities between the study sites.



# Resident and irruptive nomad golden jackals (*Canis aureus*) in a European forest-agricultural habitat

Csányi, Erika<sup>1\*</sup>; Sándor, Gyula<sup>1</sup>

<sup>1</sup> University of Sopron, Institute of Wildlife Biology and Management, Sopron, Hungary

\* 123karierika@gmail.com

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The rapid range expansion of the golden jackal (*Canis aureus*) across Europe highlights the importance of understanding its spatial ecology to inform effective population management strategies. Despite their growing presence, limited research exists on the species home range dynamics within the densely populated forest–agricultural mosaics subjected to intense hunting pressure. This study investigates variations in home range sizes and movement behaviours of golden jackals in southwestern Hungary, emphasizing differences by sex, age, and season and the occurrence of irruptive nomadism. Over two years, 45 GPS-collared jackals (23 males and 22 females) were monitored for an average of 245 days each, yielding 236,675 hourly location data points. Home range sizes were calculated using the 95% kernel home range estimator, and trajectory segmentation differentiated residents (single home range) from irruptive nomads (shifting or multiple home ranges).

Resident jackals exhibited mean (±SE) home range sizes of 14.38 ± 2.27 km<sup>2</sup> (n=28), although individual ranges varied substantially, with differences reaching up to 100-fold. Male jackals generally occupied larger home ranges than females, and juveniles had more extensive ranges than adults, especially during the pup-rearing season. Home range shifts were more frequent among juveniles compared to adults and among females compared to males. The average home range size of nomadic jackals was 92.44 ± 20.59 km<sup>2</sup> (n=17). Conversely, sex and age did not significantly influence the home range sizes of irruptive nomads. Jackals predominantly utilized forested areas near forest–agriculture interfaces and avoided man-made structures. The findings revealed substantial interindividual variation in spatial usage and pronounced intra-annual changes in home range sizes between sexes, reflecting the species' ecological adaptability. These results underscore the interplay of social structure, high population density, intense hunting pressure, the absence of large carnivores, and seasonal food availability in shaping the spatial ecology of golden jackals.



# Movement activity of red deer stags in response to hinds' oestrus cycles

Sándor, Gyula<sup>1</sup>; Csányi, Erika<sup>1\*</sup>; Tari, Tamás<sup>1</sup>

<sup>1</sup> University of Sopron, Institute of Wildlife Biology and Management, Sopron, Hungary

\* 123karierika@gmail.com

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We studied the connection between red deer stags' movement patterns and the hinds' reproductive cycles in Somogy County, Hungary. Our hypothesis proposed a link between the changes in stags' daily movement activity and the period when hinds enter oestrus. Temporal patterns of conception (n=89), including the onset, peak, and end of the mating period, were identified for the studied population. Concurrently, the stags' activity levels during this ecologically critical time frame were monitored using GPS telemetry (n=4). The findings revealed that 60% of hinds were conceived between 31 August and 19 September, 2020. A significant difference was observed between the conception rates in the early and later stages of the rutting period. During the initial phase, oestrus synchrony among hinds led to increased activity in stags due to harem herding behaviour, compared to the pre-rut period. As the rut progressed and the number of fertile hinds decreased, stags exhibited heightened movement activity, likely attributed to intensified mate-searching behaviour. Our results demonstrate that the oestrus patterns of hinds significantly influence the movement dynamics of red deer stags.



# Movement ecology of individual wild boar (*Sus scrofa*) in response to different management approaches

Dalcanale, Simone<sup>1,2,3\*</sup>; Pontiggia, Pietro<sup>3,4</sup>; DeNicola, Vickie<sup>1,2,3</sup>; Franzetti, Barbara<sup>5</sup>; Monaco, Andrea<sup>5</sup>; Michelotto, Eleonora<sup>6</sup>; Gallo, Michele<sup>6</sup>, Garret, Street<sup>7</sup>; Ossi, Federico<sup>1,8</sup>; Cagnacci, Francesca<sup>1,8</sup>

- <sup>1</sup> Fondazione Edmund Mach, San Michele all'Adige, Italy
- <sup>2</sup> University of Trento, San Michele all'Adige, Italy
- <sup>3</sup> Field Engine Wildlife Research and Management, Connecticut, USA
- <sup>4</sup> Università degli Studi di Roma Torvergata, Roma, Italy
- <sup>5</sup> Istituto Superiore per la Protezione e la Ricerca Ambientale, Roma, Italy
- <sup>6</sup> Ente Parco Regionale Colli Euganei, Padua, Italy
- <sup>7</sup> Mississippi State University, Mississippi State, USA
- <sup>8</sup> National Biodiversity Future Centre, Palermo, Italy
- \* simone.dalcanale@fmach.it

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The proliferation of wild boar populations has increased concerns about the transmission of African swine fever (ASF) from wild to domestic environments. This disease represents a serious threat to both wild and domestic pig populations, requiring extensive measures to prevent ASF contamination. In addition, the combination of intensive agricultural practices and landscape fragmentation and urbanisation has increased the human-wildlife interface, also escalating human-wild pig conflicts, with significant economic implications. In this context, the need for management methods of this highly plastic and opportunistic species has increased. Yet, little attention has been given, so far, to understanding the effect of individual removals on the movement ecology of the remaining components of the sounders.

In addition to culling and traditional trapping methods, a new trapping method based on a simple but effective trap, the Pig Brig® (Field Engine Wildlife Research and Management, FEWR), has recently been introduced. The Pig Brig<sup>®</sup> is a passive trapping system that works like a creel, exploiting the rooting behaviour of pigs. The aim of this trap is to increase the number of individuals captured in a single trapping event (aiming at the whole sounder), while reducing the impact on individuals and the cost of management operations. Our project aims to analyse the effects that removal events with different methodologies (trapping and hunting) may have on spatial behaviour of adult wild boar females. The proposed experimental design involves the use of Pig Brig<sup>®</sup> for the trapping operations, with captures run in triplets of two independent treatment groups and one control, and 10 independent repetitions in the same study area (Parco Regionale Colli Euganei, Padua, Italy). In each sounder, the largest adult female is fitted with bio-logging collars (GPS and 3D accelerometers), while other individuals are alternatively: (i) instantly culled inside the trap (Treatment 1); (ii) fitted with ear tags first and culled about 30 days after released (Treatment 2); (iii) fitted with ear tags and released with no culling operations (Control). Treatment 1 and 2, respectively, allow monitoring the effects of capture and removal using traps and hunting, with particular focus on space use and dispersal of large adult females. By doing this, we hope to highlight the consequences in terms of movement behaviour for the most commonly used management methods and therefore to define new strategies to control wild boar.



# Effect of landscape structure on European hare (*Lepus europaeus*) resting place locations

Jarkovský, František<sup>1\*</sup>; Ševčík, Richard<sup>2</sup>; Cukor, Jan<sup>1,2</sup>

<sup>1</sup> University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Prague, Czech Republic

<sup>2</sup> Forestry and Game Management Research Institute, v.v.i., Jíloviště, Czech Republic

\* jarkovsky@fld.czu.cz

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We examined the abundance and distribution of European hare resting places in various landscape structures as well as the preferred vegetation height for resting places, resting place dimensions, and their distance from field edges. Fieldwork was conducted in two sessions during spring and autumn at the Vrbová Lhota-Ratenice and Cerhenice located in the Central Bohemian Region of the Czech Republic. Resting places were monitored in 16-hectare squares divided by linear transects. These lines followed the routes of field walks conducted for data collection. Resting places were located visually. For each resting place, we recorded the GPS position, cover type (or crop species), surrounding vegetation height, and resting place dimensions. Simultaneously, field maps were prepared, depicting field shapes and cover types.

Hares preferred senescent grasses, permanent grassland, peas, and at the Vrbová Lhota-Ratenice location with smaller soil unit areas for resting places. The preferred average vegetation height for resting places was 8.2 cm in the first monitoring session in spring. In the second session, this value increased to 30.4 cm, and in the third, autumn session, it reached an average height of 23.6 cm. Hares exhibited different preferences within the monitoring areas. At the Cerhenice site, vegetation reached an average height of 25.0 cm, while at the Vrbová Lhota-Ratenice site, it reached 15.4 cm. Most recorded heights were within the 5 to 15 cm interval. Resting places at Cerhenice reached 54 m on average away from the edge of the soil unit, while at Vrbová Lhota-Ratenice, the average distance was 26.7 m. The number of resting places decreased with distance from the edge in this area, whereas in Cerhenice, their number increased. During the spring months, the average length of resting places was 37.6 cm, decreasing to 35.3 cm in autumn. The width during the spring monitoring averaged 15.9 cm, while in the autumn it reached 12.9 cm.

The results of this study, particularly regarding the preference for older vegetation, permanent grasslands, peas, and vegetation height, can be used as a basis for mitigating the impacts of agricultural intensification and for streamlining measures to improve habitat conditions for hares.



# Alone or with a companion? Cooperative behaviour of hunting dogs in driven hunts

Lazárková, Karolína<sup>1\*</sup>; Benediktová, Kateřina<sup>1</sup>; Adámková, Jana<sup>1</sup>; Bartoš, Luděk<sup>1,2</sup>; Lukáč, Martin<sup>1</sup>

<sup>1</sup> Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Department of Game Management and Wildlife Biology, Prague, Czech Republic

<sup>2</sup> Institute of Animal Science, Department of Ethology, Prague, Czech Republic

\* lazarkova@fld.czu.cz

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Cooperation among hunting dogs during driven hunts is essential for the successful execution of these hunts and the effective regulation of wild boar populations, which have been increasing across Europe. This contribution aims to evaluate cooperative behaviour among hunting dogs during driven hunts and to analyse the factors influencing cooperation between individual dogs. The importance of this work lies in understanding the effects of social bonds, training, and environmental conditions on the efficiency of hunting dogs during driven hunts.

We tested the hypothesis that hunting dogs work together in a single beat, but unrelated and/or unfamiliar dogs with one another would exhibit lower willingness to cooperate in searching for and driving game than related or familiar dogs. Using GPS technology and Garmin Virb Elite cameras we assessed cooperative behaviour by monitoring real-life situations and potential cooperation among hunting dogs. Data were collected over two hunting seasons (2019/2020 and 2020/2021) during 13 group hunting events in the hunting grounds of the Czech University of Life Sciences (ČZU). In total, 233 hunting dogs participated in these events. We observed the dogs' responses in the beat to the "initiator," i.e. the dog that first located the game and began alerting. We analysed whether and which factors influenced the willingness of other dogs to join the initiator, including age, sex, breed, distance from the initiator, familiarity with the initiator, and game species/category.

Overall, 26 events were recorded in which the initiator discovered and alerted to the game, causing prompt responses from other dogs. Analysis showed that the only significant factor affecting cooperation was the distance from the initiator; dogs closer to the initiator were more likely to join in the pursuit of the game. No significant effects on cooperation were found for familiarity among dogs, sex, age, size, or breed. This study contributes to a better understanding of the factors influencing hunting dog cooperation, which can positively impact their training and the organization of driven hunts.



# Assessment of lead and cadmium in fish species from Begečka Jama Natural Park as environmental pollution indicators

Polovinski Horvatović, Miroslava<sup>1\*</sup>; Beuković, Dejan<sup>1</sup>; Vukadinović, Marko<sup>1</sup>; Guljaš, Darko<sup>1</sup>; Damjanović, Marko<sup>1</sup>; Krstović, Saša<sup>1</sup>; Jajić, Igor<sup>1</sup>

<sup>1</sup> University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Novi Sad, Serbia

\* miroslava.polovinski@stocarstvo.edu.rs

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Heavy metals, such as lead and cadmium, are significant micropollutants originating from both natural processes and anthropogenic activities. The Begečka Jama Natural Park, spanning 379 hectares and located approximately 18 km from Novi Sad, is an ecologically important area formed from an old Danube riverbed. Despite its natural significance, the park is surrounded by intensively cultivated agricultural land and several illegal landfills, raising concerns about potential contamination. This study aimed to assess the presence of lead and cadmium in three fish species, namely *Micropterus salmonides, Carassius gibelio, and Hypophthalmichthys molitrix,* as bioindicators of environmental pollution. These fish species, which are an integral part of the local food chain and are occasionally consumed by fishermen, were analysed to determine heavy metal concentrations in their gills, liver, and caudal muscle. Water and sediment samples from several locations within the park were also tested for lead and cadmium levels before fish sampling. All analysed samples showed heavy metal concentrations within the permissible limits set by Serbian legislation. These findings indicate that, while current contamination levels are not alarming, the proximity of pollution sources necessitates continued monitoring to safeguard the park's ecological integrity and ensure the safety of its aquatic species.



### European small mammals in the agricultural landscape: an overview

Hein, Susanne<sup>1\*</sup>; Imholt, Christian<sup>1</sup>; Nopper, Joachim<sup>1</sup>; Jacob, Jens<sup>2</sup>

- <sup>1</sup> BASF SE, Agricultural Solutions, Ecotoxicology, Limburgerhof, Germany
- <sup>2</sup> Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Rodent Research, Münster, Germany
- \* susanne.hein@basf.com

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Many small mammals are keystone species in European agricultural landscapes. They are an integral part of terrestrial food webs, affecting predator species by bottom-up trophic processes through fluctuating prey availability. Burrowing small mammals, as ecosystem engineers, can alter soil structure and nutrient fluxes and provide habitat structures for other animals in their tunnel systems. As seed dispersers and predators, they shape plant recruitment in many habitats. Despite these key contributions, there is a pervasive dialectic in the perception of rodents in the public eye as well as the scientific literature. They are the most diverse and abundant mammalian order and multiannual, high-density fluctuations of widespread, generalist species like common vole (*Microtus arvalis*) can lead to high damage to crops and significant monetary losses in agriculture.

Intensively managed agricultural habitats are often characterized by reduced overall biodiversity and a homogenized vertebrate community that primarily include small mammals with high resilience to human disturbance. From the regional species pool, generalist species would utilize agricultural areas allowing them to succeed in the agricultural landscape, while others cannot. The degree of tolerance to agricultural practice depends on a multitude of factors including but not limited to niche or diet breadth, number of offspring, nest placement (i.e., below- or aboveground) or dispersal strategies in relation to landscape composition. Different degrees of tolerance impact community composition, population dynamics and long-term presence in a particular area. Specific traits determine if species will avoid or benefit from agricultural landscapes in the long term.

So far, the focus of small mammal research in agricultural settings has mainly been on only a few species, typically the most prominent pest species in agricultural production. However, large-scale assessments of community level changes are still scarce for small mammals. Therefore, we propose to bring together experts in the field of small mammal research from all over Europe to collect expertise of regional small mammal communities. In a combined analysis, our aim is to identify universal species traits to describe the degree of species' avoidance, tolerance or dependence on agricultural land.



## Synchronous masting regulates seed-seed interactions for two cofruiting tree species under the mediation of rodents

Wang, Jing<sup>1</sup>; Zhang, Bo<sup>2</sup>; Han, Ning<sup>1</sup>; Feng, Tuo<sup>1</sup>; Hou, Xiang<sup>1</sup>; Zhao, Jidong<sup>1</sup>; An, Xiaolei<sup>1</sup>; Chen, Xiaoning<sup>1</sup>; Chang, Gang<sup>1\*</sup>

<sup>1</sup> Shaanxi Institute of Zoology, Shaanxi Key Laboratory of Qinling Ecological Security, Xi'an, China

<sup>2</sup> Shangluo University, Shangluo, China

\* changgang@xab.ac.cn

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Knowledge is lacking on how masting regulates seed-seed interactions of sympatric tree species under the mediation of rodents. Here, we compared rodent-mediated seed dispersal between two sympatric tree species (*Castanea mollissima* and *Quercus aliena*) in both monospecific and mixed plots, across five successive years in the Qinling Mountains, China. We investigated: (a) whether the rodentmediated seed dispersal success of each tree species was affected by the presence of the other, and (b) if any effects on dispersal were influenced by either the abundance of rodents present, or by (c) masting (i.e., seed abundance). We found both short-term mutualism and competition occurred between these two sympatric tree species. In non-mast years, the presence of *Q. aliena* seed reduced predation on *C. mollissima* seeds and promoted seed caching, which resulted in higher rates of *C. mollissima* seed dispersal. Synchronous masting enhanced this benefit to *C. mollissima* seeds, although we detected no clear neighbour or masting benefits for *Q. aliena* seed dispersal. This shows that interspecific interactions differ between these masting neighbours, which may relate to either relative species-specific seed abundance or seed traits. We conclude that seed abundance mediates rodents' seed foraging and caching strategies, which may be important for forest regeneration and forest tree species composition.



# Fuel source shift or cost reduction: context-dependent adaptation strategies in closely related *Neodon fuscus* and *Lasiopodomys brandtii* against hypoxia

Li, Xiujuan<sup>1+</sup>; Qiao, Congcong<sup>1+</sup>; Chen, Bojian<sup>2,3+</sup>; Li, Mengyang<sup>1</sup>; Chen, Peng<sup>4,5</sup>; Huang, Maolin<sup>1</sup>; Chen, Chunxiao<sup>1</sup>; Liu, Yan<sup>1</sup>; Cheng, Han<sup>1</sup>; Jiang, Mengwan<sup>6</sup>; Shi, Luye<sup>1,4\*</sup>; Wang, Zhenlong<sup>1\*</sup>

<sup>1</sup> Zhengzhou University, School of Life Sciences, Zhengzhou, China

<sup>2</sup> Tongji University, College of Environmental Science and Engineering, Shanghai, China

<sup>3</sup> Jiaxing-Tongji Environmental Research Institute, Jiaxing, China

- <sup>4</sup> Kunming Institute of Zoology, Chinese Academy of Sciences, State Key Laboratory of Genetic Resources and Evolution, Kunming, China
- <sup>5</sup> University of Chinese Academy of Sciences, Kunming College of Life Science, Kunming, China
- <sup>6</sup> Henan University of Technology, School of Artificial Intelligence and Big Data, Zhengzhou, China
- \* wzl@zzu.edu.cn (Z. Wang), sly@zzu.edu.cn (L. Shi)
- + Authors contributed equally to this work

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Oxygen is essential for most life forms. Insufficient oxygen supply can disrupt homeostasis and compromise survival, and hypoxia-induced cardiovascular failure is fatal in many animals, including humans. However, certain species have adapted and evolved to cope with hypoxic environments and are therefore good models for studying the regulatory mechanisms underlying responses to hypoxia. Here, we explored the physiological and molecular responses of the cardiovascular system in two closely related hypoxia-adapted species with different life histories, namely, Qinghai voles (*Neodon fuscus*) and Brandt's voles (*Lasiopodomys brandtii*), under hypoxic (10% O<sub>2</sub> for 48 h) and normoxic (20.9% O<sub>2</sub> for 48 h) exposure. Kunming mice (*Mus musculus*) were used for comparison. Qinghai voles live in plateau areas under hypoxic conditions, whereas Brandt's voles only experience periodic hypoxia.

Histological and hematological analyses indicated a strong tolerance to hypoxia in both species, but significant cardiac tissue damage and increased blood circulation resistance in mice exposed to hypoxia. Comparative transcriptome analysis revealed enhanced oxygen transport efficiency as a coping mechanism against hypoxia in both *N. fuscus* and *L. brandtii*, but with some differences. Specifically, *N. fuscus* showed up-regulated expression of genes related to accelerated cardiac contraction and angiogenesis, whereas *L. brandtii* showed significant up-regulation of erythropoiesis-related genes. Synchronized up-regulation of hemoglobin synthesis-related genes was observed in both species. In addition, differences in cardiometabolic strategies against hypoxia were observed in the rodents. Notably, *M. musculus* relied on adenosine triphosphate (ATP) generation via fatty acid oxidation, whereas *N. fuscus* shifted energy production to glucose oxidation under hypoxic conditions and *L. brandtii* have evolved different adaptation strategies to enhance oxygen transport capacity and conserve energy under hypoxia. Our findings suggest that the coping mechanisms underlying hypoxia tolerance in these closely related species are context dependent.



# Comparative study on auditory physiological structure and function between the mandarin vole and the Brandt's vole

Yang, Xuanlin<sup>1</sup>; Li, Qinghua<sup>1\*</sup>

<sup>1</sup> Zhengzhou University, School of Life Sciences, Zhengzhou, China

\* liqinghua@zzu.edu.cn

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Mammals' auditory capabilities are influenced by social behaviour, habitat, and developmental stages. Research on rodent hearing has evolved from studying morphology to cochlear mechanisms and molecular levels. Current studies on surface-active rodents primarily examine age-related hearing loss and the effects of high-altitude hypoxia on auditory function. In contrast, research on subterranean rodents focuses on their low-frequency hearing mechanisms. These rodents, living in dark underground burrows, have adapted their communication and auditory physiology to their environment, favouring mid-low frequency sounds while being less sensitive to high frequencies compared to surface-active rodents. For example, the specialized auditory structures in subterranean rodents may compensate for diminished other senses. By comparing the auditory abilities, structural differences, and molecular mechanisms of hearing between subterranean and surface-active rodents, we can understand the adaptability and changes in auditory systems across different environments, offering insights into the evolution and function of these systems.

The present study compares the auditory physiological differences between a subterranean rodent, the mandarin vole (Lasiopodomys mandarinus), and a surface-active rodent, the Brandt's vole (Lasiopodomys brandtii), to elucidate the adaptive evolution of their auditory systems to unique acoustic environments. Auditory capabilities were assessed using Auditory Brainstem Response (ABR) tests. The results showed that the auditory ranges of the mandarin vole and the Brandt's vole are similar, with overlapping sensitive frequency ranges, and the most sensitive frequencies lie between 5000 and 7000 Hz. The average auditory thresholds of the mandarin vole in the 500-2000 Hz and 16000-24000 Hz frequency bands are comparable to those of the Brandt's vole. However, significant differences were observed: the click threshold of the mandarin vole is significantly higher than that of the Brandt's vole; the auditory thresholds of the mandarin vole at frequencies between 4000 and 12000 Hz are significantly higher than those of the Brandt's vole; and the average auditory thresholds of the mandarin vole between 2000 and 16000 Hz are higher than those of the Brandt's vole. These findings suggest that the mandarin vole has weaker auditory capabilities than the Brandt's vole, possibly indicating auditory degeneration during the adaptive evolution to the subterranean environment. Comparison of the tympanic bulla structures revealed that the mandarin vole has a shorter tympanic bulla diameter, smaller tympanic bulla spacing, and a narrower external width compared to the Brandt's vole. Additionally, the tympanic bulla of the Brandt's vole is more fragile with a thinner external wall. Micro-CT and histological section observations of the cochlear structures showed typical spiral configurations in both species, with the cochlea of the mandarin vole being flatter than that of the Brandt's vole.



# Collection of pest management data used to improve rat control in Denmark

Søndergaard, Kirsten<sup>1\*</sup>; Christiansen, Henrik Svaneberg<sup>1</sup>

<sup>1</sup> Danish Environmental Protection Agency, Water Resources, Odense, Denmark

\* kis@mst.dk

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The Danish legislation (the Danish Statutory Order on prevention and control of rats) has made it mandatory for citizens to report actual or suspected occurrences of rats to the local authorities. The rat control is most often carried out by the local authority – for free, but citizens can also choose to use a private PCO, this however is not free of charge and will be covered by the citizen. Whether rat control is done by the local authority or a private PCO, there is a requirement that rat control may only be carried out by an authorized person. When carrying out rat control, the authorized person must make a mandatory report (online). The reported data are automatically transferred to a central database and here the data are available for local authorities and DEPA. The database is owned by DEPA and all the Danish municipalities. DEPA and the municipalities supervise that all rat control (the municipal or the private) comply with this requirement. This gives opportunity to extract various and adequate data for the control of rats in Denmark, at local and national level and use these data to suggest improvement for rat control in general.

Here we present examples of data that provide detailed and valid information about: (i) the development in reports of rat occurrences from 2015 until 2024; (ii) the most common causes to rat occurrences; (iii) the development in use of chemical and non-chemical control methods; (iv) variation of reports over the year, and between rural and urban areas.



# A catalogue of anomalies and rare traits of dentition of arvicoline rodents: implications for monitoring of lemming populations in the Arctic

Markova, Evgenia<sup>1\*</sup>

<sup>1</sup> Institute of Plant & Animal Ecology UrB RAS, Ekaterinburg, Russia

\* e.markova@ipae.uran.ru

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Recent studies have shown that global warming threatens populations of lemmings in the Low Arctic, thus emphasizing the need for species monitoring. A catalogue of congenital and acquired pathologies and rare traits of dentition of arvicoline rodents is created based on the analysis of dental variation in natural populations of widespread North-Eurasian voles and lemmings (*Lemmus, Myopus, Dicrostonyx, Arvicola, Clethrionomys, Lasiopodonys, Alexandromys, Micortus*) and in laboratory colonies of *Dicrostonyx* and *Microtus*. It is shown that anomalies and pathologies of dentition in arvicolines are non-species- or genus-specific and the same approach might be used to assess phenotypic disorders for most taxa in Arvicolinae. A scale for assessing the phenotypic severity of dental disorders in arvicolines is developed, which allows identifying phenotypically vulnerable populations and determining possible detrimental factors for further testing.

Application of the approach is illustrated by the example of the Siberian lemmings (*Lemmus sibiricus*) and Palearctic collared lemmings (Dicrostonyx torquatus) sampled in the north of Western and Eastern Siberia. In the averaged data, 99.9% of individuals of the Siberian and 95% of collared lemmings are phenotypically normal, i.e., they have <1 point on the scale of acquired disorders and 0 points on the scale of congenital anomalies. At the boundaries of the species' ranges and on islands, congenital anomalies of dentition appear as extremely rare traits in Siberian lemming, and accumulate (up to 20%) in collared lemming. The maximum number of different types of congenital anomalies occurring simultaneously in one wild population in Siberian lemming is <1 (on average, it tends to 0), and in collard lemming <3 (on average, 1). With inbreeding in the laboratory, the total scores of aberrations in collared lemming can reach 7 per colony. The scores can be used as comparative values for assessing the severity of congenital abnormalities of dentition at either individual or population levels. Analysis of the occurrence of acquired pathologies within the modern ranges of the two species shows that this category of features is extremely rare in Siberian lemming (with the exception of minor intravital breakage of tooth prisms). In collared lemming, acquired disorders associated with oral diseases (caries-like and actinomycosis-like lesions) are found in one natural population in Yakutia. These disorders have been also traced in the laboratory colony, the founders of which were taken from the same population. Caries-like lesions in this colony were found in 1-3 animals in each laboratory generation from F1 to F9, in two individuals in F9 caries-like lesions were associated with the bone tissue damage. The results obtained indicate that in the Palearctic collared lemming, dental anomalies are more common than in Siberian lemming and they are not eliminated by population dynamics throughout the range of collared lemming showing a tendency to increase in frequency over time.

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# Proposed methodology for monitoring the endemic Balkan snow vole (*Dinaromys bogdanovi*)

Lisičić, Duje<sup>1</sup>; Čuljak, Dora<sup>2</sup>; Huten, Tea<sup>3</sup>; Bjedov, Linda<sup>4\*</sup>; Lugić, Edin<sup>5</sup>; Boljfetić, Marko<sup>4</sup>; Petković, Monika<sup>2</sup>; Hamidović, Daniela<sup>6</sup>; Kryštufek, Boris<sup>7</sup>; Tvrtković, Nikola<sup>8</sup>

- <sup>1</sup> University of Zagreb, Faculty of Science, Zagreb, Croatia
- <sup>2</sup> Oikon Ltd. Institute of Applied Ecology, Zagreb, Croatia
- <sup>3</sup> Supernatural Ltd., Zagreb, Croatia
- <sup>4</sup> University of Zagreb, Faculty of Forestry and Wood Technology, Zagreb, Croatia
- <sup>5</sup> Elytron, Zagreb, Croatia
- <sup>6</sup> Ministry of Environmental Protection and Green Transition, Institute for Environment and Nature, Zagreb, Croatia
- <sup>7</sup> Slovenian Museum of Natural History, Ljubljana, Slovenia
- <sup>8</sup> Association Natura Society for Nature Protection of Croatia, Zagreb, Croatia
- \* lbjedov@sumfak.unizg.hr

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Balkan snow vole (*Dinaromys bogdanovi*) is a glacial relict endemic to the Dinaric Mountain range in the Eastern Adriatic. It is a habitat specialist associated with karst topography characterized by deep stone fissures, crevices, caves, and sinkholes. The Balkan snow vole is a secretive and neophobic species, making the conventional live trapping methodology extremely time-consuming; consequently, its biology remains understudied. According to the IUCN, Balkan snow vole is categorized as a Vulnerable species (VU), while in Croatia, it was listed as Data Deficient (DD) species and is strictly protected. Since this species is included in Appendix IV of the EU Habitats Directive, Croatia is required to report on its conservation status to the European Commission.

Therefore, as part of the Operational Program Competitiveness and Cohesion 2014-2020, the Republic of Croatia was developing a national monitoring program for the Balkan snow vole. Due to its secretive nature and the lack of data, the primary objective of this project was to develop an effective method for assessing the occurrence of the Balkan snow vole in its natural habitat. Camera trapping had already been used on a limited local scale; however, during this project, we applied the method across the entire distribution range of the species in Croatia. Our primary aim was to evaluate this methodology as a potentially easy and effective means of detecting the presence of the target species. While camera trapping demonstrated both advantages and disadvantages, it ultimately proved to be the most effective approach for assessing *D. bogdanovi* occurrence in its natural habitat.



# Analysis of the real growth rate of brown hare (*Lepus europaeus*) in different hunting grounds of the Republic of Serbia in 2024

Mihajlović, Nikola<sup>1\*</sup>; Stepić, Stefan<sup>1</sup>; Lavadinović, Vukan<sup>2</sup>; Beuković, Dejan<sup>3</sup>; Ignjatović, Aleksandar<sup>1</sup>; Popović, Zoran<sup>1</sup>

<sup>1</sup> University of Belgrade, Faculty of Agriculture, Belgrade, Serbia

<sup>2</sup> University of Belgrade, Faculty of Forestry, Belgrade, Serbia

<sup>3</sup> University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

\* nikola.mihajlovic@agrif.bg.ac.rs

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Brown/European hare (*Lepus europaeus*) is one of the most important species of small game, both in Europe and in the Republic of Serbia. However, it faces a continuous decline in population numbers due to the complex influence of natural and anthropogenic factors. Effective management of this species requires not only monitoring population numbers but also conducting a detailed analysis of age structure, which enables the assessment of the real growth (increment) rate. Based on the real growth rate as well as data on population size and previous management practices recorded by hunting associations, recommendations for the next hunting season can be formulated.

The aim of this study was to determine the real growth rate of brown hares in 2024 at various localities across the Republic of Serbia, with hares sampled from a total of 15 hunting grounds. The analysis included a total of 969 individuals. The age of each individual was determined based on the lens weight, allowing for a precise assessment of the population's age structure. Based on the determined age, the real growth rate, reflecting the proportion of young individuals (up to one year old) in the total population, was calculated. The real growth rates varied significantly across hunting grounds, ranging from 23.5% to 65.2%. The observed real growth rates for the hunting grounds were as follows: 59.9%, 55.0%, 53.2%, 60.0%, 34.8%, 54.5%, 23.5%, 41.9%, 29.7%, 59.4%, 62.7%, 53.1%, 41.7%, 62.7% and 65.2%, respectively. Based on these values and data provided by hunting associations, recommendations were made for the subsequent hunting season. In five hunting grounds (33.3%), hunting was recommended according to the management plan; in another five hunting grounds, a complete suspension of hunting was requested.

Differences in real growth rates among hunting grounds can be explained by geographical and climatic factors, as the hunting grounds are located in different regions of the Republic of Serbia. Additionally, the level of agricultural development varies across the observed hunting grounds, influencing food availability and, consequently, reproduction and the real growth rate of brown hare. Furthermore, in some of the observed hunting grounds, the real growth rate had been monitored in previous years, while in others this was not the case. It is possible that consistent monitoring of the real growth rate over an extended period and the application of hunting recommendations contributed to better results in certain hunting grounds.

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# Distribution and population abundance of stone partridge (*Alectoris graeca*) in Croatia during the last decade

Tomljanović, Kristijan<sup>1</sup>; Ančić, Mario<sup>1</sup>; Vodopija, Valerija<sup>1</sup>; Vucelja, Marko<sup>1</sup>; Bjedov, Linda<sup>1\*</sup>

<sup>1</sup> University of Zagreb, Faculty of Forestry and Wood Technology, Zagreb, Croatia

\* lbjedov@sumfak.unizg.hr

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Stone partridge (Alectoris graeca Meisner) is a widespread bird species in the mountainous region of Europe and Asia. It is native to Croatia inhabiting the coastal area of rocky terrain overgrown with the degradation stages of deciduous and coniferous forests. In Croatia, it is listed and managed as a game species. The study of population dynamics was carried out in seven counties which are part of the stone partridge distribution range. After the Second World War, the population in Croatia was estimated at 7,000 individuals with a growing trend. In the 1970s and 1980s, the population reached its peak and the number was estimated at 35,000 individuals. This was followed by a decline in numbers, and in 2010 the population was estimated at 11,000 individuals. For a more detailed analysis, this research processed data segmentally by county for the period 2010-2020. The data was available from hunting management plans including 211 hunting grounds. Data showed that the most suitable areas are in the central part of Dalmatia. In the northern and central part of Dalmatia, average density was 4-14 individuals/100 ha. However, further to the south the abundance of stone partridge increases reaching the average density of 1-19 individuals/100ha. In the area of the north-western coastal part of Croatia, stone partridge has a significantly lower average density of 0.1-6 individuals/100 ha. The situation is similar in the area below the Velebit mountain range where an average density of 4-10 individuals was recorded. During the research period (2010-2020), the population trend did not change significantly, so it can be concluded that the population is in stagnation. Possible factors for this could be increased abundance of predators (mustelids), loss of grasslands (pastures) due to abandonment of traditional animal husbandry as well as increased urbanization.



### Helping each other: *Falco vespertinus* population and ecosystem services in Parma agricultural landscape

Gustin, Marco<sup>1</sup>; Scaravelli, Dino<sup>2,3\*</sup>

<sup>1</sup> Conservation Department LIPU-Birdlife Italia, Parma, Italy

<sup>2</sup> University of Bologna, BIGEA Department, Bologna, Italy

<sup>3</sup> STERNA scarl, Forlì, Italy

\* dino.scaravelli@unibo.it

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Red-footed falcon (*Falco vespertinus*) is a vulnerable species by IUCN Red List and a globally threatened species of European conservation concern (SPEC 1). Actually over 90% of the Italian population bred in the Parma province of the Emilia-Romagna region. An extensive conservation program started in 2010 providing artificial nests, and the number of boxes has grown from 63 in that year to 190 in 2024. The number of pairs has therefore increased from 21 to 134 in the last year. Reproductive success was from 1.4 to 2.8 in the 2010-2024 period. This conservation and monitoring effort has allowed a notable increase in the population and has completely changed the presence of the species in northern Italy, due to the source effect shown in the progressive increase of the other populations present in the Po Valley.

Considering the findings about the diet of this population, with a strong propensity to consume voles and insects present in the fields and in the marginal areas of crops, the importance of preserving an agricultural landscape mainly dedicated to the cultivation of high-nutritional-value forage to feed dairy cows (supporting the local production of Parmigiano Reggiano cheese) has been verified. In the area, apart from the red-footed falcon populations there are also many pairs of other falcon species (i.e., *Falco tinnunculus* and *Falco naumanni*) as well as nocturnal species with similar niche (e.g. *Tyto alba, Strix aluco, Athene noctua*). This is largely due to both the effort to provide artificial shelters and to maintain agricultural spaces attentive to these species, having on the other hand the great ecosystem value of controlling rodent species and insects that could cause crop damage.



### Plovers under pressure: assessing the role of tourism and dune management in the nest site of Algerian coast

Bakhouche, Badis<sup>1</sup>; Boulaouad, Belkacem Aimene<sup>2</sup>; Djemadi, Imed<sup>3</sup>; Rezouani, Imad Eddine<sup>4</sup>; Selmane, Chabani<sup>1</sup>; Dino, Scaravelli<sup>5\*</sup>

- <sup>2</sup> Mohamed el Bachir el Ibrahimi University, Department of Agronomy, Bordj Bou Arreridj, Algeria
- <sup>3</sup> Environment Research Center, Environment and Biodiversity Division, Annaba, Algeria
- <sup>4</sup> École Normale Supérieure de Kouba, Laboratory of Eco-Biology Animals (LEBA), Kouba, Algeria
- <sup>5</sup> University of Bologna, BIGEA Department, Bologna, Italy
- \* dino.scaravelli@unibo.it

#### DOI: 10.20315/evmc.2025.124

Plovers are considered a group of endangered species globally and particularly in the Mediterranean area. Their location of nests in dunes and open lands makes them not only easily attacked by predators but, above all, make them sensitive to the ever-increasing use of this particular environment by humans. To verify the state of conservation of these species and in the context of studies aimed at clarifying the reproductive success and behaviour of *Caradius* species in coastal areas of Algeria, we have been monitoring for several years (with classical techniques) the presence of nests and the success of nesting in the Le Sablettes sites in Algiers. The species recorded were Kentish plover (*Anarhynchus alexandrinus*; KP) and little ringed plover (*Caradius dubius*; LRP).

The difference in the number of nests found during the covid-19 period, when the beaches were not frequently visited by people, and what was subsequently detected, is very significant. In 2020, in Promenade des Sablette 15 KP and 18 LRP plover nests were recorded, and in 2021 those figures were 22 for KP and 24 for LRP, respectively. Unfortunately, in 2022 only 9 nests were found of the two species that have been abandoned following intensive "development work" carried out without any pre-assessment of its consequences. Only 2 KP nests were saved thanks to an early hatching, i.e., before the start of the works. In 2023, tourists started to intensively use the area and we were able to find only 5 nests of KP that were unfortunately predated by gulls. Tourist attendance, now very intense after the "modernization" of the area, increases the pressure over the nests, and adults move away due to disturbance, increasing the risk of predation and thermal imbalance.

Proposals have been suggested for the conservation of the oviposition sites and in particular the maintenance of non-frequented areas, but with poor results at the moment. The lack of sensitivity and surveillance are the elements most influential on the disturbance, and the associations and researchers are engaging with the respective local authorities not only by counting/monitoring but also by raising awareness *in situ* as well as in the press, trying to stop the rapid decline of both studied species.



<sup>&</sup>lt;sup>1</sup> University of Science and Technology Houari Boumediene, Department of Ecology and Environment, Algiers, Algeria

### Preliminary assessment of loggerhead sea turtle (*Caretta caretta*) overlap with industrial fisheries in the Adriatic Sea

Jančič, Matic<sup>1,2</sup>; DeCarolis, Gemma<sup>3</sup>; McKillip, Sara<sup>3</sup>; McWeeny, Molly<sup>3</sup>; Zekan, Mateja<sup>2</sup>; Lazar, Bojan<sup>1,4\*</sup>

<sup>2</sup> Blue World Institute of Marine Research and Conservation, Veli Lošinj, Croatia

<sup>3</sup> School for Field Studies, Center for Marine Megafauna, Veli Lošinj, Croatia

<sup>4</sup> Juraj Dobrila University of Pula, Faculty of Natural Sciences, Marine Sciences Program, Pula, Croatia

\* bojan.lazar@upr.si

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Loggerhead sea turtles (*Caretta caretta*) are the most abundant marine turtle species in the Mediterranean. Due to recent increases in nest numbers, the Mediterranean regional management unit has been delisted by the IUCN to the status of Least Concern, but remains conservation dependent. Incidental catch in both passive and active fishing gear is considered to be the biggest threat to sea turtles in marine habitats. In the Mediterranean, industrial fisheries represent a small fraction of the fleet (~13%) but are responsible for ~89,000 catches and ~17,500 deaths per year. Within the Mediterranean, the Adriatic Sea is one of the most important foraging and overwintering grounds for loggerhead turtles, utilised year-round by individuals of different life stages. Small juveniles consume both pelagic and benthic prey, retaining plasticity in habitat use, while large juveniles and adults predominantly forage on the sea bottom. For all life stages, spatial distribution and diving patterns depend on thermoregulation and shift in accordance with the seasonal sea surface temperature (SST) changes in the Adriatic Sea. These life history traits influence exposure and susceptibility of loggerhead turtles to bycatch.

As the Adriatic Sea is heavily exploited by industrial fisheries, this study aims to identify areas with the highest overlap of sea turtles and industrial fisheries, interaction 'hotspots', and analyse changes across seasons and life stages to advance understanding of spatiotemporal bycatch risk in the Adriatic Sea. We obtained space use data from 21 satellite tagged loggerhead turtles, tracked between 2014 and 2024. Location data was filtered in order to remove invalid locations and locations with large error estimates, and analysed separately for small juveniles (n=11) and large juveniles and adults (n=10). Fisheries data was obtained from European Marine Observation and Data Network in the form of monthly aggregated vessel densities (h/km<sup>2</sup>) for the period 2017-2023. We split locations and fisheries data according to temperature changes in the Adriatic Sea into cold (Nov-Apr; SST range 13.5-18.0°C) and warm season (May-Oct; SST range 17.7-26.2°C). The overlap was quantified as the number of turtle locations multiplied by fisheries data within 10x10 km grid and rescaled to obtain index values between 0 (no interaction) and 1 (highest relative interaction). Our results show that the overlap between turtles of both life stages and fishing vessels was concentrated in the northern Adriatic in the warm season, then shifted southwards and extended into the central Adriatic during the cold. Interaction hotspots were identified in the Gulf of Venice and south of the River Po delta during the warm season, and off the Istrian peninsula year-round. These preliminary results provide a basis to inform targeted onboard monitoring programs and improve sea turtle bycatch mitigation in the Adriatic Sea.



<sup>&</sup>lt;sup>1</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Department of Biodiversity, Koper, Slovenia

# Continuous monitoring of golden jackal: a case study at Lake Cerknica, Slovenia

Prusnik, Lara<sup>1\*</sup>; Zupan, Sara<sup>1</sup>; Kraševec, Rudi<sup>2</sup>

<sup>2</sup> Notranjska Regional Park, Cerknica, Slovenia

\* l.prusnik@gmail.com

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From January to the end of May 2024 we conducted monitoring of golden jackal (*Canis aureus*) in the area of Lake Cerknica. Lake Cerknica is the largest Slovenian intermittent lake, characterized by the transition between aquatic and terrestrial ecosystems and rich biodiversity. Golden jackal has been permanently present in Slovenia since 2009. The aim of this study was to determine the distribution and abundance of jackals in the area of Lake Cerknica. We used the playback method of jackal calls according to the bioacoustics method protocol to determine the number of territorial groups in the area. Using QGIS 3.36.2, we plotted the data on a map and determined that six of the nine calling stations were suitable for further monitoring. There are 5-6 jackal groups in the Lake Cerknica area, with group sizes ranging from two to seven individuals. Based on the conducted survey and water level data we indicated where jackals could be at the time of monitoring. We propose to conduct permanent monitoring of jackals in this area by using the six most suitable calling stations and following the standard protocol of the bioacoustic method.



<sup>&</sup>lt;sup>1</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Department of Biodiversity, Koper, Slovenia

# Monitoring insights for advanced conservation and management of large carnivores

Fedorca, Mihai<sup>1,2\*</sup>

<sup>1</sup> Transilvania University of Brasov, Brasov, Romania

<sup>2</sup> National Institute for Research and Development in Forestry "Marin Dracea", Brasov, Romania

\* mihai.fedorca@yahoo.com

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Monitoring of wildlife has become a key focus in recent years, requiring high-quality data to assess population status, species interactions, habitat use, and breeding patterns, particularly for elusive species. Photo-traps (cameras) have emerged as a valuable tool in wildlife research, offering new insights into species behaviour and individual movements. Our study was conducted across 11 hunting grounds in Buzău and Prahova counties, situated in the mountainous and hilly regions of the southeastern Carpathians. Over the past two years, we deployed 55 photo-traps from three different models. Brown bear (Ursus arctos) was selected as a target species. To determine optimal camera placement, we first implemented preliminary monitoring methods, including tracking in snow/mud and identifying marking signs. Once installed, cameras remained in the field for different time intervals to assess the impact of trap-days on detection rates. The collected data, consisting of photos and/or videos, were stored internally. Two camera models allowed for remote data downloads, while one required manual extraction of the memory card. Data analysis revealed notable differences between the two counties, including the number of identified individuals, the months with the highest activity, the time of day when bears were the most active, and the total number of species recorded. These findings contribute to a better understanding of brown bear ecology and provide a foundation for improving monitoring strategies and conservation efforts in the region.



### Real-time monitoring of brown bear behaviour: integrating Deep Learning pose estimation with remote hair snare sampling in the Catalan Pyrenees

Campanera-Moline, Arnau<sup>1\*</sup>; Sazatornil, Victor<sup>1</sup>; Ponsa, Daniel<sup>2,3</sup>; Benavente, Robert<sup>2,3</sup>; Lumbreras, Felipe<sup>2,3</sup>; Ramilo-Henry, Maguiña<sup>1</sup>; Caparroz, Noel<sup>1</sup>; Brotons, Lluís<sup>1,4,5</sup>

- <sup>2</sup> Computer Vision Center, Bellaterra (Cerdanyola del Vallès), Spain
- <sup>3</sup> Universitat Autònoma de Barcelona (UAB), Department of Computer Science, Bellaterra (Cerdanyola del Vallès), Spain
- <sup>4</sup> Center for Ecological Research and Forestry Applications, Cerdanyola, Spain
- <sup>5</sup> Spanish National Research Council, Madrid, Spain
- \* arnau.campanera@ctfc.cat

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Advances in wildlife monitoring technologies have made identifying animal species, individuals, and tracking their movements more efficient and cost-effective. Camera traps and hair snares are widely used noninvasive tools in wildlife conservation, particularly for monitoring and studying bear populations. However, in remote areas where 4G-connected camera traps are unavailable, conservationists often rely on inefficient methods, such as blindly visiting traps, risking cross-contamination of samples and wasting valuable time and resources. In recent years, an increasing number of researchers are turning to Machine and Deep Learning (ML; DL) to monitor biodiversity. Pose estimation tools based on DL can allow conservationists to estimate animal postures and infer behaviours such as rubbing.

Here, we propose a DL-based pose estimation model trained from 1,326 images of brown bear (*Ursus arctos*) collected in the Catalan Pyrenees. It is based on the YOLOv11 pose estimator and is intended to be integrated into edge devices with satellite communication capabilities monitoring hair snares in remote areas. This real-time system has the potential to enhance the collection of samples by guiding conservationists solely to visited snares and reducing the delay between visits and sample collection, making the monitoring practices in remote areas much more efficient.



<sup>&</sup>lt;sup>1</sup> Forest Science and Technology Center of Catalonia (CTFC), Landscape Dynamics and Biodiversity, Solsona, Spain

# Monitoring of the Balkan chamois (*Rupicapra rupicapra balcanica*) populations in the territory of the Republic of Srpska

Gačić, Dragan<sup>1,3\*</sup>; Zubić, Goran<sup>2</sup>; Mrđenović, Damir<sup>3</sup>; Jovanović, Milanka<sup>2</sup>, Bjelić, Diana<sup>4</sup>

<sup>1</sup> University of Belgrade, Faculty of Forestry, Belgrade, Serbia

<sup>3</sup> University of Banja Luka, Faculty of Forestry, Banja Luka, Bosnia and Herzegovina

<sup>4</sup> Public enterprise "Forests of the Republic of Srpska", Sokolac, Bosnia and Herzegovina

\* dragan.gacic@sfb.bg.ac.rs

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Balkan chamois (Rupicapra rupicapra balcanica) is an important indigenous large game species in the Republic of Srpska. This constitutional-legal unit covers around 2.46 million hectares, which corresponds to around 49% of the area of Bosnia and Herzegovina. There are three types of hunting grounds in the Republic of Srpska: (i) special hunting grounds; (ii) sports and recreational hunting grounds; and (iii) commercial hunting grounds. In terms of number and total area, sports and recreational hunting grounds, which were given to hunting associations for management, dominate (N=78) and cover approximately 2.17 million hectares, which corresponds to 88% of the total area of hunting grounds in the Republic of Srpska. The aim of this study is to analyse the dynamics of the number and spatial distribution of Balkan chamois populations in the hunting grounds of the Republic of Srpska in the period 2007-2023. We used data collected by the Ministry of Agriculture, Forestry and Water Management for each hunting year, namely the estimated spring count, the culling plan, and the realized culling. The spatial distribution was determined on the basis of field research conducted as a part of the international project Biopolis/2023/009 CPRV AO2 (i.e., a case study of the Horizon project Biodiversity Genomics Europe, BGE). We included 12 hunting grounds: Bakić, Panos, Čemernica, Vrbnica, Treskavica, Velež, Sjemeć, Šipovo, Zelengora, Sušica, Kamenica, and Gacko. The map of the spatial distribution of chamois was created using a 1×1 km grid in the ArcGIS 10.3 software package.

The main factors threatening populations of Balkan chamois in the Republic of Srpska are illegal hunting (poaching), habitat loss, and fragmentation. During the period 2007-2023, the total area suitable for the Balkan chamois decreased (668 vs. 604 km<sup>-</sup>). It was noted that there is no aggregated and detailed data for a longer period at the level of geographic regions and the constitutional-legal entity (e.g. trophies, sex and age structure of hunted individuals, recorded losses). The estimated spring abundance had an increasing trend in the period 2007-2023 (approx. 1,100 vs. 1,500 individuals), but is still below the habitat potential, as the estimated optimal number would be >2,600 individuals. The most numerous and biologically and economically valuable population is located in the special hunting ground Zelengora, managed by the National Park Sutjeska, where 1,079 individuals were estimated in spring 2023, which is about 70% of the total number in the Republic of Srpska. It has been noted that in recent years the Balkan chamois has spontaneously spread from existing hunting grounds to new suitable habitats in the vicinity (e.g. Mount Romania and Drina Canyon). It can be concluded that the current monitoring system and protection against poaching should be significantly improved, in particular through cooperation between the competent institutions and the users of hunting grounds (both hunting associations and public enterprises).



<sup>&</sup>lt;sup>2</sup> Ministry of Agriculture, Forestry and Water Management, Department of Forestry, Hunting and Wood Processing, Banja Luka, Bosnia and Herzegovina

# Natural and anthropogenic factors influencing the Tatra chamois (*Rupicapra rupicapra tatrica*) in the Nízke Tatry (Low Tatras) Mts. (Central Slovakia)

Hrdý, Tomáš<sup>1\*</sup>; Hronček, Pavel<sup>2</sup>; Hrončeková Gregorová, Bohuslava<sup>2</sup>; Urban, Peter<sup>1</sup>

- <sup>1</sup> Matej Bel University, Faculty of Natural Sciences, Department of Biology and Environmental Studies, Banská Bystrica, Slovakia
- <sup>2</sup> Matej Bel University, Faculty of Natural Sciences, Department of Geography and Geology, Banská Bystrica, Slovakia
- \* thrdy@umb.sk

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Tatra chamois (*Rupicapra rupicapra tatrica*, Blahout 1972) is a rare glacial relict and endemic mammal subspecies. An autochthonous population of this ungulate occurs in the alpine zone of the Tatra Mts., in their Slovak and Polish parts. In geographical terms, local populations represent the northern most natural limit of the species' range in Europe. As the Tatra chamois subspecies may be considered an evolutionarily significant unit it requires conservation management. The native chamois population became extinct in the Low Tatras in consequence of natural climatic changes in the mid-Holocene (about 10,000 years ago). However, anthropogenic deforestation has once again created suitable conditions for its redistribution in the western part of the mountain. Reintroduction of the Tatra chamois from the Tatras to the Low Tatras Mts. took place in the years 1969-1976. In total, 30 chamois were released into the wild during this conservation translocation. This founding population has increased in number since release to the current 130-160 individuals.

The chamois population in this mountain range, which has been a national park since 1978, is threatened by several natural and anthropogenic factors. Of the natural factors, in addition to predators and avalanches, it is mainly the gradual shift of the upper krummholz-line to higher mountain altitudes mostly due to succession. The period of grazing boom that took almost five centuries ended in the second half of the 20th century (1960-1970s) when there was a gradual decline in sheep and cattle grazing on alpine pastures. After 1989, there was literally no grazing left on the national park pastures.

Hybridization between the Alpine chamois (*R. r. rupicapra*) and the *R. r. tatrica* is a major concern for the Low Tatras population. Genetic analysis indicates that interbreeding has occurred. Conservationists' proposals to remove the Alpine chamois as an alien form, which is a legal game, have been resisted because of conflict with hunting interests.

In addition, the centre of the chamois area is divided by cable cars, lifts and ski slopes of the Jasná resort (Chopok - north and Chopok - south), which were established in the middle of the last century and have been significantly expanded since 2010. The main anthropogenic impact for Tatra chamois in the Low Tatras is a higher intensity of tourism, winter free (off-piste) skiing, ski mountaineering, motorized and non-motorized airplanes and paragliding, etc. Activities involving overnight camping away from designated huts, and dog-walking, are thought to be particularly disturbing, especially during rutting, birthing, and nursing seasons.



### Roe deer hunting between facts, traditions and beliefs

Müller, Michael G.1\*; Jordan-Fragstein, Claudia C.1

<sup>1</sup> TUD Dresden University of Technology, Chair of Forest Protection, Tharandt, Germany

\* michael.mueller@tu-dresden.de

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European roe deer (*Capreolus capreolus*) is the most widespread wildlife species in Germany and therefore a subject of hunting in nearly all habitats. Due to its importance, forest owners and hunters are interested in successful hunting regimes, which depend on their respective objectives. However, objectives of forest owners and hunters are not always in accordance and sometimes even oppositional. Hunters are often confused by old traditions and beliefs. Furthermore, hunting regimes often contradict current scientific knowledge of wildlife biology and ecology – as well as the hunters' objectives. In addition, most forest owners are not educated in forest sciences and thus do not have sufficient knowledge of silviculture, forest products, and forest damage in their ownership. The goal of this contribution is to compare different trials of hunting regimes taking into account recent scientific studies and to present conclusions for wildlife management and roe deer hunting.



# Efficacy of wildlife deterrents in minimizing white-tailed deer consumption of bait used for attracting wild boar

Kupferman, Caitlin A.<sup>1,2\*</sup>; DeNicola, Anthony J.<sup>3</sup>; Sumrall, Aaron<sup>4</sup>; Pontiggia, Pietro<sup>5</sup>; Beasley, James C.<sup>1,2</sup>

<sup>1</sup> University of Georgia, Savannah River Ecology Laboratory, Aiken, SC, USA

<sup>2</sup> University of Georgia, Warnell School of Forestry and Natural Resources, Athens, GA, USA

<sup>3</sup> White Buffalo, Inc., Moodus, CT, USA

- <sup>4</sup> Pig Brig Trap Systems, Moodus, CT, USA
- <sup>5</sup> Pig Brig Trap Systems, Italy

\* ckupferman@uga.edu

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Wild boar (Sus scrofa) is a globally distributed species that causes extensive damage to native plant and animal communities throughout their native and introduced ranges. Trapping is a widely used method for controlling populations of wild boar (native range) and wild pigs (introduced range) that has been shown to be effective in reducing damages. When trapping, individuals are typically captured using traps baited with whole-kernel corn or similar baits. However, these bait sites often serve as attractants to non-target species including cervids (e.g., white-tailed deer [Odocoileus virginianus]), which may consume substantial portions of bait and reduce the likelihood of wild boar visitations, ultimately interfering with trap success. Thus, research is needed to identify baits and/or deterrents that optimize wild boar and wild pig visits while limiting non-target species consumption of bait. Using remote cameras paired with bait stations treated with commercially available wildlife deterrents (bloodmeal, natural capsaicin, synthetic capsaicin, and Liquid Fence®), we examined the efficacy of wildlife deterrents in repelling deer while maintaining wild pig visitations. We found that deer consumed corn less frequently at sites treated with liquid fence, bloodmeal, and natural capsaicin than controls, with the most pronounced response at sites treated with liquid fence and natural capsaicin. Wild pig visitations to bait stations were similar between controls and all treatments with the exception of our lowest concentration of synthetic capsaicin, suggesting the treatments evaluated were effective at reducing deer visitations while maintaining consumption by wild pigs. This research provides valuable information regarding the effectiveness of wildlife deterrents, aiding in effective wild boar and wild pig management.



# Assessing short-term behavioural impacts of capture on wild boar: implications for wildlife monitoring and management

Masilkova, Michaela<sup>1\*</sup>; Hrazánek, Ivo<sup>2</sup>; Ježek, Miloš<sup>1</sup>

<sup>2</sup> University of South Bohemia in České Budějovice, Department of Zoology, České Budějovice, Czech Republic

\* masilkova@fld.czu.cz

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Monitoring and GPS tracking of animals are crucial to understand their ecology and thus informing wildlife management practices. Capture, immobilisation and manipulation of free-ranging animals to equip them with GPS collars might negatively affect their behaviour for several days after capture and thus bias the data. The magnitude and duration of these detrimental effects depend on the studied species, sex, season, and type of used trap. This study aimed to assess the short-term behavioural impacts of capture and GPS collaring on wild boar (*Sus scrofa*; n = 108) movement and activity, as well as the influence of sex, age, trap type, and season. After trapping, the movement and activity of wild boar were significantly lower, but the effects were, in general, small. The latency to recover, however, depended on the studied variable. While the activity score and daily used area (90% minimum convex polygon) were gradually restored after 7 days, the daily distance from the trap was restored after 12 days. We further found that younger (juvenile and subadult) individuals and wild boar trapped in wooden corral traps had larger daily used areas and that males were moving further away from traps. These findings highlight the importance of accounting for short-term post-capture behavioural changes when interpreting GPS tracking data and provide insights for designing more effective and minimally invasive wildlife monitoring protocols.



<sup>&</sup>lt;sup>1</sup> Czech University of Life Sciences Prague, Department of Game Management and Wildlife Biology, Prague, Czech Republic

### A challenging decade for wild boar populations in Europe: African swine fever and different management strategies have driven diverse trends across the continent

Barukčić, Valentina<sup>1\*</sup>; Pokorny, Boštjan<sup>2,3</sup>; Cerri, Jacopo<sup>4</sup>; Hackländer, Klaus<sup>5</sup>; Bego, Ferdinand<sup>6</sup>; Licoppe, Alain<sup>7</sup>; Casaer, Jim<sup>8</sup>; Ballian, Dalibor<sup>9,3</sup>; Stoyanov, Stoyan<sup>10</sup>; Ježek, Miloš<sup>11</sup>; Håkansson, Bo<sup>12</sup>; Kunnasranta, Mervi<sup>13</sup>; Miettinen, Elmo<sup>13</sup>; Keuling, Oliver<sup>14</sup>; Papakostas, Konstantinos<sup>15</sup>; Náhlik, András<sup>16,24</sup>; Ozoliņš, Janis<sup>17</sup>; Belova, Olgirda<sup>18</sup>; Cellina, Sandra<sup>19</sup>; Janković, Miloš<sup>20</sup>; de Nijs, Nils<sup>21</sup>; Zimmermann, Barbara<sup>22</sup>; Podgórski, Tomasz<sup>4,11</sup>; Fonseca, Carlos<sup>23</sup>; Farkas, Attila<sup>24</sup>; Markov, Nickolay<sup>25</sup>; Gačić, Dragan<sup>26</sup>; Lazár, Petar<sup>27</sup>; Colomer, Joana<sup>28,29</sup>; Berqvist, Göran<sup>30</sup>; Fischer, Claude<sup>31</sup>; Šprem, Nikica<sup>1</sup>

<sup>1</sup>University of Zagreb, Faculty of Agriculture, Zagreb, Croatia; <sup>2</sup>Faculty of Environmental Protection, Velenje, Slovenia; <sup>3</sup>Slovenian Forestry Institute, Ljubljana, Slovenia; <sup>4</sup>Mammal Research Institute, Białowieża, Poland; <sup>5</sup>University of Natural Resources and Life Sciences, Vienna, Austria; <sup>6</sup>University of Tirana, Tiranë, Albania; <sup>7</sup>Public Service of Wallonia, Gembloux, Belgium; <sup>8</sup>Research Institute for Nature and Forest, Brussels, Belgium; <sup>9</sup>University of Sarajevo, Sarajevo, Bosnia and Herzegovina; <sup>10</sup>University of Forestry, Sofia, Bulgaria; <sup>11</sup>Czech University of Life Sciences Prague, Praha, Czech Republic; <sup>12</sup>Danish Society for Nature Conservation, København, Denmark; <sup>13</sup>Natural Resources Institute Finland, Helsinki, Finland; <sup>14</sup>University of Veterinary Medicine Hannover, Hannover, Germany; <sup>15</sup>University of Ioannina, Ioannina, Greece; <sup>16</sup>University of Sopron, Sopron, Hungary; <sup>17</sup>Latvian State Forest Research Institute Silava, Salaspils, Latvia; <sup>18</sup>Lithuanian Research Centre for Agriculture and Forestry, Kedainiu, Lithuania; <sup>19</sup>Musée national d'histoire naturelle Luxembourg, Luxembourg; <sup>20</sup>Ministry of Agriculture, Forestry and Water Management, Podgorica, Montenegro; <sup>21</sup>Faunabeheereenheid Noord-Brabant, the Netherlands; <sup>22</sup>University of Inland Norway, Elverum, Norway; <sup>23</sup>University of Aveiro, Aveiro, Portugal; <sup>24</sup>Sapientia Hungarian University of Transylvania, Sfântu Gheorghe, Romania; <sup>25</sup>Institute of Plant and Animal Ecology, Yekaterinburg, Russia; <sup>26</sup>University of Belgrade, Belgrade, Serbia; <sup>27</sup>University of Veterinary Medicine and Pharmacy, Košice, Slovakia; <sup>28</sup>Minuartia, Barcelona, Spain; <sup>29</sup>University of Barcelona, Barcelona, Spain; <sup>30</sup>Swedish Association for Hunting and Wildlife Management, Nyköping, Sweden; <sup>31</sup>Haute Ecole du Paysage, d'Ingénierie et d'Architecture, Genève, Switzerland \* vbarukcic@agr.hr

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As a cosmopolitan species, wild boar (Sus scrofa) population density has been increasing till recent times across Europe, as a result of environmental changes as well as due to favourable biological and ecological characteristics and life-history traits of the species. The fact that wild boar has a very high reproductive potential and that it is an omnivorous species with very diverse food habits may have contributed to the rapid spread of the species around the globe. The increase of wild boar abundance has various impacts on the economy and the environment through the destruction of agricultural land, traffic collisions, and health risks as the growth of wild boar populations has the potential to spread infections and zoonoses. Recently, wild boar and domestic pigs have been affected by a highly contagious viral disease, African swine fever (ASF). The disease appeared in Europe in 2014 in the Baltic countries and Poland, from where it spread to the west. The disease per se, but also accompanied by different mitigation measures (mainly focused on lethal measures, i.e., more intensive hunting actions, also by using several modern tools for increasing hunting efficiency as well as to motivate hunters for culling as many wild boar as possible), have affected previously consistent increasing trend of wild boar, i.e., they have caused recently diverse trends across the continent. We analysed the hunting bags of wild boar from almost all European countries from 2000 to 2024 and calculated among-countries variability in population trends to determine whether and how the occurrence of ASF and the various management strategies used to control the disease have affected the wild boar population in Europe.



### Wild fauna conservation and increasing outdoor recreational activities: how to coexist?

Mariet, Anne-Lise<sup>1\*</sup>; Bellefleur, Clément<sup>2</sup>; André, Antoine<sup>2</sup>; Sage, Mickaël<sup>1</sup>

<sup>1</sup> Faune INNOV' R&D, Besançon, France

<sup>2</sup> Parc Naturel Régional des Ballons des Vosges, Munster, France

\* anne-lise.mariet@faune-innov.fr

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Outdoor recreational activities have expanded significantly in recent decades due to four key factors: (i) diversification of activities, (ii) greater accessibility through technical advancements, (iii) proliferation of routes enabled by GPS tracking and social networks, and (iv) a growing urban population seeking connection with nature. While these activities bring notable social and economic benefits, they also increase human disturbance affecting wild fauna. Research highlights the impacts on sensitive species including behavioural changes (e.g. fight or flight response), physiological stress, and reduced fitness and reproduction success. In Europe, most studies have focused on winter disturbance in high-altitude areas (e.g. skiing, snowshoeing), leaving gaps in knowledge about other activities and contexts, such as plains, wetlands or low-altitude forests. To mitigate such impacts, "tranquillity areas" have been established across Europe, supported by regulatory or voluntary initiatives. These areas rely on awareness campaigns to educate outdoor enthusiasts about minimizing wildlife disturbance. However, their effectiveness remains poorly evaluated, with limited studies on their acceptability and success, especially outside winter context (e.g. Phasianidae species).

Within the LIFE20 IPE/FR/0019 program, an innovative method was developed to assess tranquillity areas in the Grand Est region of France that presents varied ecological contexts and outdoor recreational activities. This approach includes: (i) surveys to gauge acceptance among recreational users and tourism professionals, and (ii) the dashboard providing a standardized monitoring tool adaptable to various ecosystems and activities. Preliminary data are being collected and this presentation will address: (a) current knowledge about human-wildlife interactions, (b) the tranquillity area concept in Europe, and (c) initial insights from the proposed assessment method.



# Working with stakeholders to mitigate a human-wildlife conflict: challenges and opportunities

Stauder, Julia<sup>1\*</sup>; Kostner, Benjamin<sup>1</sup>

<sup>1</sup> Eurac Research, Institute for Regional Development, Bozen, Italy \*julia.stauder@eurac.edu

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After centuries of absence, grey wolf (Canis lupus) has returned to the Alps, supported by strong protection laws that have enabled its population to recover. However, the species' prolonged absence has disrupted traditional coexistence practices, leading to significant conflicts, particularly with livestock farmers. The European LIFEstockProtect project aims to mitigate these human-wildlife conflicts by promoting practical protective measures, including the use of livestock guardian dogs, electric fencing, and permanent shepherding. The project has implemented various activities, such as peer-to-peer training courses for breeders and shepherds, educational videos on livestock guardian dogs, field excursions showcasing best practices, public awareness campaigns, and the establishment of a volunteer network to assist shepherds in the field. A central component of LIFEstockProtect is stakeholder engagement, an approach widely recognized for enhancing ownership and fostering mutual understanding between decision-makers and those impacted by their decisions. In the context of human-wildlife conflicts, stakeholder involvement is vital for identifying and addressing both real and perceived barriers to implementing effective protection measures. This presentation will focus on the Italian project region of South Tyrol, where wolves have been gradually increasing since their return in 2010, alongside a rise in livestock predation. Despite this, acceptance of wolves and willingness to adopt protective measures remain low, particularly among livestock farmers who bear the brunt of these challenges. Throughout the project, diverse approaches were employed to collaborate and communicate with this key stakeholder group to reduce conflict and develop sustainable solutions. We will present challenges faced during these efforts, share successful strategies, and open a discussion on effective methods for engaging stakeholders in addressing contentious issues like human-wildlife coexistence.



#### Engaging key stakeholders for lowering conflicts between humans and wolves: insights and results from the LIFE WolfAlps EU project

Omizzolo, Andrea<sup>1\*</sup>; Chioso, Christian<sup>2</sup>; Moroni, Agnese<sup>1</sup>; Favilli, Filippo<sup>1</sup>; Stauder, Julia<sup>1</sup>; Kostner, Benjamin<sup>1</sup>

<sup>1</sup> Eurac Research, Institute for Regional Development, Bolzano, Italy

<sup>2</sup> Valle d'Aosta Region, Department of Natural Resources and Forestry Corps, Aosta, Italy

\* andrea.omizzolo@eurac.edu

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The recently concluded European LIFE WolfAlps EU project was focused on guaranteeing the long-term conservation of the Alpine wolf population by improving wolf-human coexistence. One of the main actions throughout the 5-year project period was the involvement of key actors who interact in different ways with the wolf such as breeders, hunters, environmental associations, politicians, journalists, hikers, tour operators and educators, with the aim to explore the economic, political and social aspects of the wolf presence in the Alpine territories. Its influence on community life is directly related to the amount of space that humans and wolves share. Therefore, following European guidelines, the project organised 11 dialogue thematic (regional and local) platforms and a very large number of local meetings tailored for each stakeholder in all project's countries.

Here we will present the main findings of these participatory paths, which were attended by more than 3,000 people. First, the adopted engagement strategy will be explained and its key points briefly presented. Afterwards, urgent issues and needs of the participants according to the local context, the relevant issues for each stakeholder's category, the main common discussion points and feedback to the project and the activities locally implemented, strengths, challenges, recurrent problematic issues and the main ideas and suggestions on innovative actions and/or strategies for managing wildlife conflicts, supporting pastoral activities, and engaging the public will be briefly illustrated. We will discuss what the project has done to contribute to the advancement of knowledge on this sensitive topic.



### A risk assessment framework for badger and beaver burrowing in Anthropogenic landscapes: implications for infrastructure

Seljee, Ferdi<sup>1\*</sup>; Ward, Sophie<sup>1</sup>; Sijtsma, Ilse<sup>1</sup>; Smink, Teun<sup>1</sup>; de Jong, Ronald<sup>1</sup>

<sup>1</sup> Altenburg & Wymenga Ecological Research LLC, Human-Wildlife Conflict Team, Veenwouden, the Netherlands \* f.seljee@altwym.nl

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In recent decades, populations of Eurasian beaver (*Castor fiber*) and European badger (*Meles meles*) have significantly increased in the Netherlands, leading to a rise in human-wildlife conflicts. In low-lying and highly anthropogenic areas, the burrowing behaviour of these species can pose significant safety risks to dikes and the foundations of infrastructure. Burrowing is therefore a major concern for railway managers and water authorities, among others. The Dutch railway network is one of the densest and busiest in Europe, with much of it constructed on elevated track formations made of relatively soft sediment. With approximately three thousand kilometres of railway track to manage, railway authorities must identify and prioritise the most vulnerable sections to effectively mitigate risks. We developed a risk assessment tool based on habitat suitability modelling and quantification of burrowing risk, which we applied to the Dutch railway system. In this model, habitat suitability is derived from a spatial analysis of land-use data, habitat data, and remote sensing data of woody vegetation. In our approach, burrowing risk is determined by factors such as the proximity to suitable habitat and water (for beavers), the dimensions of the track formation, and the presence of burrowing-resistant fortifications, among others. A spatial analysis of the railway network prompted field surveys of identified risk areas, revealing several previously undocumented beaver and badger sites. This risk assessment method could serve as a basis for long-term monitoring and management plans to minimise human-wildlife conflicts in anthropogenic landscapes and can be applied to various types of infrastructure.



### Spatiotemporal patterns of wildlife-vehicle collisions on the Slovenian highways

Al Sayegh Petkovšek, Samar<sup>1\*</sup>; Kotnik, Klemen<sup>1</sup>; Breznik, Kristijan<sup>1,2</sup>; Pokorny, Boštjan<sup>1,3</sup>

<sup>1</sup> Faculty of Environmental Protection, Velenje, Slovenia

<sup>2</sup> International School for Social and Business Studies, Celje, Slovenia

<sup>3</sup> Slovenian Forestry Institute, Ljubljana, Slovenia

\* samar.petkovsek@fvo.si

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We studied species composition, monthly patterns and linear collision hotspots (using KDE+ methodology) of roadkill of large and medium sized mammal species (European roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), wild boar (*Sus scrofa*), brown bear (*Ursus arctos*), golden jackal (*Canis aureus*), red fox (*Vulpes vulpes*), European badger (*Meles meles*), and brown hare (*Lepus europaeus*)) along 778 km of Slovenian fenced highways in the period 2018-2020. In the three-year period, we registered in total 2046 roadkill cases, with red fox (N=790; 39.6%), roe deer (N=592; 28.9%), and European badger (N=423; 20.7%) being the most exposed species. The finding that red fox was the most vulnerable mammal species considering highway roadkill strongly contradicts to registered wildlife roadkill on all Slovenian roads (including highways), where the number of road-killed roe deer in the same period (2018-2020) was fourfold higher in comparison with the roadkill of red fox. The high rate of red fox roadkill found in our study suggests their ability to access highways below or through damaged fences, which is consistent with findings from other Central European countries as well as with our own field inspection at Slovenian highways. On the other hand, fences were quite effective in keeping ungulates out of the highways.

We analysed the monthly distribution of roadkill for four species (roe deer, red fox, European badger, brown hare), which accounted for 97% of recorded vertebrate roadkill on highways. Temporal collision patterns with evident species-specific monthly peaks reflect the behaviour and activity of studied species, however with some exceptions (roe deer, badger) compared to previous studies. For example, we observed a significant variation in the monthly distribution of roe deer roadkill, with a pronounced peak in spring, but secondary peak in summer/autumn was not registered at Slovenian highways. Obviously, fences along highways effectively block roe deer wandering across highways in summer and autumn, while they are not so effective during much more intensive spring movements due to social restructuring, i.e. establishing territories, or dispersion of yearlings. Further, bimodal pattern of roadkill of badger at Slovenian highways was not detected, showing that either adults reproducing in summer/autumn or the later-born offspring have different movement behaviour, i.e. they have lower need to cross highways in comparison with the springtime. The yearly roadkill of red fox shows bimodal pattern: the two peaks occur in January and during late summer to autumn. Reported roadkill of brown hare was the least frequent and has almost uniform temporal distribution across months.

We identified 268 KDE+ hotspots, accounting for 48.5% of all roadkill and covering 9.3% (72.6 km) of the highway network. Recognising these hotspots is crucial for implementing the most effective mitigation measures to reduce wildlife vehicle collisions. Indeed, the identified hotspot locations may enable decision-makers to select and implement the most effective mitigation strategies.



### NPR: A decision support tool for selecting wildlife mitigation measures

Nezval, Vojtěch<sup>1\*</sup>; Bíl, Michal<sup>1</sup>; Kušta, Tomáš<sup>2</sup>; Keken, Zdeněk<sup>3</sup>

<sup>1</sup> CDV – Transport Research Centre, Department of Geoinformatics, Brno, Czech Republic

<sup>2</sup> Czech University of Life Sciences Prague, Department of Game Management and Wildlife Biology, Prague, Czech Republic

<sup>3</sup> Czech University of Life Sciences Prague, Department of Applied Ecology, Prague, Czech Republic

\* vojtech.nezval@cdv.cz

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Wildlife vehicle collisions (WVC) occur on roads all over the world. In Czechia alone, several thousand WVCs are recorded every year. Since WVCs represent a serious threat for road traffic safety, several mitigation measures have been invented. These include, for example, fences, odour repellents, acoustic devices or wildlife warning signs. Individual measures differ in their parameters, efficiency, price and suitability regarding the given place. Road managers have to select both effective and affordable measures which are appropriate for the roads under their administration. The decision-making process is not, however, always in line with the recent findings from relevant research. This means that inappropriate measures (in terms of effectiveness or overall price) are often implemented.

In order to help the stakeholders, involved in the process of planning, approval, and implementation of measures to reduce WVC, we developed a decision support tool (NPR). It is accessible at https://npr.cdvinfo.cz. In the first part, the tool contains information on the most frequently used WVC mitigation measures in Czechia, their pros and cons, recommendations for their application, and links to the relevant scientific literature. In the second part, the user can specify the input parameters, such as the type of considered WVC measure (e.g. fencing, odour repellents, wildlife-warning reflectors), road length, and traffic intensity. Then, the tool automatically evaluates the suitability and benefit of the measure over the longer term. The tool is primarily used by managers of secondary roads who do not have the option to install fencing. Additionally, it is utilized by game management authorities aiming to reduce wildlife casualties on roads. The main benefit of the tool is its cost-benefit analysis, which helps eliminate the deployment of certain measures. Similarly, if any parameter of a given location, such as traffic intensity, reaches high values, the tool will recommend specific measures. With each analysis, the tool informs users about the necessity of maintenance and monitoring of the selected measures. Outputs from the decision support tool can be downloaded in .pdf format for further discussion or comparison of different variants.



#### Ecological peace corridors to conserve human and biological diversity

Cazzolla Gatti, Roberto<sup>1\*</sup>

\* roberto.cazzollagatti@unibo.it

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Ecological peace corridors (EPCs), a new conservation strategy, are a crucial tool for preserving biodiversity and ensuring peacekeeping. These corridors can facilitate wildlife movement and genetic exchange, counteracting the fragmentation caused by human activities. In a highly anthropized world with increasing conflicts and a global call for expanding protected areas, we must re-evaluate the importance of corridors. The importance of EPCs will grow as habitat fragmentation increases, highlighting the need for proactive conservation strategies and sustainable land-use planning. Considering also the global call for the 30x30 initiative to expand protected areas and the efforts towards rewilding and land-sharing strategies together with the renowned interest in buffer zones, the EPC framework can help reduce conflicts by providing neutral spaces, thus enhancing safety for humans and wildlife. These efforts can boost habitat connectivity, genetic diversity, and ecosystem resilience, aligning with global conservation goals. EPCs, by promoting biodiversity preservation and geopolitical stability, offer a comprehensive solution to challenges like biodiversity loss, climate change, and human conflict. Their successful implementation requires international cooperation, long-term planning, and a commitment to both ecological and human well-being.

In the contribution, I will suggest some ideas for identifying EPCs with the help of AI-ML in combination with a system similar to the Italian zonation of National Parks as a model for planning EPCs, balancing conservation and human needs.

EPCs can address environmental and geopolitical challenges in conflict zones by removing military infrastructures, restoring vegetation, establishing patrolled corridors, fostering biodiversity conservation and peacebuilding. This approach not only benefits ecosystems and wildlife but also promotes cooperation and trust among neighbouring countries, paving the way for sustainable development and peace in conflict-affected areas.



<sup>&</sup>lt;sup>1</sup> University of Bologna, Department of Biological, Geological, and Environmental Sciences, OHAI Research Hub and BIOME Lab, Bologna, Italy

### Modern drone technology as a tool for wildlife density assessment and estimation of wildlife impact

Jordan-Fragstein, Claudia C.1\*; Müller, Michael G.1

<sup>1</sup> TUD Dresden University of Technology, Chair of Forest Protection, Tharandt, Germany

\* claudia.jordan-fragstein@tu-dresden.de

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The current consequences of anthropogenic climate change are particularly evident when considering its impact on forests. Various extreme weather events are affecting forests, weakening their functions and resilience. This makes it all the more important to strengthen natural, close-to-nature forest ecosystems to align them with their ecological goals, such as diversity, resilience, adaptability, dynamic stability, and regeneration capacity. The desired forest restructuring is delayed or even hindered by excessively high wildlife densities. Regarding the questions of how much wildlife is present in a forest area and how much wildlife a forest can tolerate, or at what wildlife density silvicultural goals become achievable, opinions among different stakeholders diverge significantly. Conflicts often arise between various forest users when it comes to topics such as wildlife population levels and the goals of forest owners. Various factors, such as the increasingly mild winters and changes in habitats, are particularly benefiting large ungulates in many areas, leading to a continuous population increase over the past two decades. Wild animals are part of the ecosystem. The goal is to have "adapted wildlife populations" that align with habitat capacities. These populations are considered adapted when there is no need to protect the main tree species.

In addition to numerous traditional methods, the use of modern drone technology (unmanned aircraft systems; UAS) has been established in recent years as a precise and cost-effective tool for determining the abundance of wildlife. Using two study areas in Germany, we will demonstrate how drone-based wildlife density assessments can be used to derive conclusions about the intensity of wildlife impact.



### TRAPPER: an open-source web application for managing camera trapping and citizen science projects

Stachowicz, Izabela<sup>1,2,3</sup>; Bubnicki, Jakub<sup>2,4\*</sup>

<sup>1</sup> Open Science Conservation Fund, Białowieża, Poland

- <sup>2</sup> University of Łodź, Faculty of Biology and Environmental Protection, Department of Geobotany and Plant Ecology, Łodź, Poland
- <sup>3</sup> Instituto Venezolano de Investigaciones Científicas, Laboratorio de Biología de Organismos, Altos de Pipe, Venezuela
- <sup>4</sup> Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland

\* kbubnicki@os-conservation.org

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Camera trapping has become a vital tool in ecological research, but the exponential growth of camera trap data presents significant challenges in managing, analysing, and sharing large and complex datasets. To address these challenges, we developed TRAPPER, an innovative, fully open-source web application tailored to the demands of modern biodiversity monitoring. TRAPPER supports the analysis of both videos and images, incorporates advanced spatial filtering and web-mapping tools, and allows flexible implementation of custom data protocols to meet diverse project needs. Its newly designed, user-friendly dashboard welcomes and actively engages citizen scientists, making participation intuitive and accessible. A standout feature of TRAPPER is its integration of AI tools like MegaDetector for automated detection of animals, humans, vehicles, and empty frames, alongside support for custom species-specific models such as DeepFaune. Additionally, the platform provides robust human annotation functionalities, enabling high-precision data validation. TRAPPER is designed to streamline data sharing and interoperability by implementing the Camtrap DP standard, a key framework for standardized data exchange in camera trap projects, already utilized by GBIF.

TRAPPER's multi-user and role-based structure facilitates collaborative work on camera trapping projects among researchers, NGOs, and empowering citizen scientists. The platform has already been widely adopted by institutions and organizations across Europe, with increasing uptake in South America. By addressing critical challenges in data management and promoting open science practices, TRAPPER offers a transformative solution for advancing biodiversity research and conservation efforts.



### Utilising environmental DNA, camera traps and citizen science for effective wildlife monitoring

Duniš, Luka<sup>1</sup>; Zirkelbach, Lan<sup>1</sup>; Velkavrh, Žiga<sup>1</sup>; Bončina, Aja<sup>1</sup>; Gerič, Urška<sup>1</sup>; Pokorny, Boštjan<sup>2,3</sup>; Bužan, Elena<sup>1,2\*</sup>

<sup>1</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia

<sup>2</sup> Faculty of Environmental Protection, Velenje, Slovenia

<sup>3</sup> Slovenian Forestry Institute, Ljubljana, Slovenia

\* elena.buzan@upr.si

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Science-based monitoring of wildlife is crucial for conservation and long-term sustainable population management. The combination of different methodological approaches such as environmental DNA (eDNA) analysis, camera trap records, and citizen science involvement creates an effective and comprehensive approach for wildlife monitoring. However, citizen science data can be biased by human presence and influence on the animals being observed, leading to altered behaviours or estimation of species distribution. On the contrary, environmental DNA and camera traps do not depend on direct sightings of animals, are non-invasive, and enhance verifiable records of the presence of various species. Furthermore, eDNA determination improves wildlife monitoring, biodiversity, and risk assessment since identification may be obtained from various environmental samples (e.g. water, soil and air samples for determining species presence, including pathogens, reservoirs and vectors of diseases).

We sampled diverse forest and meadow water bodies, such as rivers, streams, ponds, and puddles in the south-western part of Slovenia. Our goal was to detect mammalian species present in the studied areas, that either use or cross these water sources using a metabarcoding approach. Then we compared the ability to detect mammalian species using eDNA approach, camera traps and citizen science app SRNA in the Rižana and Strunjan hunting grounds (near Koper, Slovenia), in 2023 and 2024. Camera traps were deployed to autonomously record images and videos of wildlife present in the area in proximity to the water bodies for a period of six months.

Our results showed that camera traps were successful in identifying large vertebrate species (e.g. ungulates) and medium sized ones, such as red fox and European hare; on the contrary, they underestimated the diversity of small mammals. Metabarcoding of eDNA from water sources resulted in higher detection of mammals (and other vertebrates), but does not provide additional information important for species viability and condition, such as health status and population abundance (the number of individuals that were present at/near the water body), which was achieved by utilising camera traps (i.e., by REM method) and/or citizen science app. We can conclude that it is important to combine different detection methods/tools to obtain comprehensive and reliable estimates about local mammal/vertebrate biodiversity.



# Spider webs, soil or leaf swabs: what are the best substrates to detect terrestrial vertebrate eDNA?

Berard, Aloïs<sup>1\*</sup>; Pradel, Julien<sup>1</sup>; Charbonnel, Nathalie<sup>1</sup>; Galan, Maxime<sup>1</sup>

<sup>1</sup> National Research Institute for Agriculture, Food and Environment (INRAE), CBGP, Montpellier, France

\* aloisberard@outlook.fr; alois.berard@inrae.fr

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Human activities have led to widespread population declines and extinctions among terrestrial vertebrates. In this situation, effective monitoring is more critical than ever to track species distribution and guide conservation and restoration actions. Recently, environmental DNA (eDNA) metabarcoding has emerged as a promising and cost-effective tool for the simultaneous detection of multiple taxa. However, low detection rates have hindered the widespread adoption of a single substrate for terrestrial vertebrates eDNA surveys. Substrate selection has been shown to strongly influence detection success across taxa, yet few studies have compared their effectiveness. As such, most research in this area remains at the proof-of-concept stage, lacking empirical foundation needed for broader application.

Our study aims to evaluate and compare several promising substrates—spider webs, soil and leaf swabs—that are cost-effective and easily collectable for large scale eDNA monitoring. Specifically, we examined community overlaps among substrates and their effects on vertebrate detection probabilities. We analysed 120 samples collected from the Landes Forest, a managed, species-poor temperate forest in Western France, and included additional samples from the Montpellier zoo to validate our detection capabilities. After sequence filtering, we identified 59 wild vertebrate taxa using 12SV5 primers and 43 with 16Smam primers across forest samples. Leaf swabs and spider webs consistently and significantly yielded a higher number of taxa per sample than soil, with spider webs producing the highest average detections using the 12SV5 primers.

These findings highlight eDNA metabarcoding as an efficient and cost-effective tool for monitoring terrestrial vertebrates, and advocate for spider webs and leaf swabs as optimal substrates for maximizing detection probabilities in rapid sampling contexts. Additionally, our results emphasize the need for further comparative studies on substrate efficiency to optimize terrestrial vertebrate detection through eDNA.



### eDNA metabarcoding of wild boar diet: a tool for local biodiversity assessment of understudied taxa of fungi and invertebrates

Bončina, Aja<sup>1\*</sup>; Sollitto, Marco<sup>1</sup>; Pokorny, Boštjan<sup>2,3</sup>; Šprem, Nikica<sup>4</sup>; Bužan, Elena<sup>1,2</sup>

- <sup>2</sup> Faculty of Environmental Protection, Velenje, Slovenia
- <sup>3</sup> Slovenian Forestry Institute, Ljubljana, Slovenia
- <sup>4</sup> University of Zagreb, Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Zagreb, Croatia

\* aja.boncina@famnit.upr.si

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Faeces from omnivorous terrestrial mammals are considered important "biodiversity capsules" and a complementary tool for improving local biodiversity assessments. Investigating the diet of opportunistic feeders such as wild boar (*Sus scrofa*) by using DNA metabarcoding provides important insights into local biodiversity, including cryptic species that are difficult to monitor using traditional methods. The opportunity to study biodiversity with DNA metabarcoding provides many advantages as follows: higher resolution of identification, higher detection probabilities of rare, elusive and quickly digested items, and the ability to sample in various environments. Moreover, in the case of studying wild boar diet, we also gain deeper insights into the ecological role of the species, trophic interactions, and the potential causes and consequences of rooting behaviour, which can all contribute to improving management decisions.

We used fresh faecal and/or gastrointestinal samples (n = 37) of wild boar, collected in central parts of Slovenia and Croatia, to investigate diet as well as local biodiversity of two of the most understudied taxonomic groups: invertebrates and fungi. We considered wild boar diet to serve as a potential indicator of local biodiversity for various reasons: (i) wild boar inhabit relatively large and diverse habitats; (ii) they have opportunistic feeding strategy, consuming a broad range of taxa available at specific periods and/or locations; (iii) there are known to root due to searching for several fungal and invertebrate taxa present in soil. We compared the influence of season and individual factors (sex and age) of individuals to variability in diet composition. The 157-bp long mitochondrial COI for barcoding invertebrates and 150-350-bp nuclear ITS1 universal primers for barcoding fungi were used. We detected 175 fungal genera (>270 species) and 114 invertebrate genera (>120 species) in faecal samples. Results showed seasonal variations in detected fungal and invertebrate genera in investigated faecal samples. Metabarcoding of wild boar faeces revealed great applicability especially for detecting microfungi, with the most frequently detected genera belonging to Candida, Pichia and Pilidium. We have also successfully detected several arthropods, especially ground beetles (e.g. Carabus, Abax, Harpalus, and Poecilus), which are considered as important food source for wild boar, but also many other insects (e.g. Lucilla, Drosophylla, Tipula, Culex) as well as some other taxa, important as food source for wild boar (e.g. flatworms, earthworms, and slugs). However, to get better insight into dietary preferences of wild boar in relation to season and location as well as the potential effect of sex/age on diet selection the dataset should be enlarged both considering geographical aspect and number of samples per area.



<sup>&</sup>lt;sup>1</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia

#### From genotype to ecotype in European roe deer

Bužan, Elena<sup>1,2</sup>; Pokorny, Boštjan<sup>2,3</sup>; Komel, Tilen<sup>1\*</sup>; Cidilko, Stefan<sup>1</sup>; Korzekwa, Anna<sup>4</sup>

- <sup>2</sup> Faculty of Environmental Protection, Velenje, Slovenia
- <sup>3</sup> Slovenian Forestry Institute, Ljubljana, Slovenia
- <sup>4</sup> Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

\* tilen.komel@upr.si

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As a widespread ungulate, European roe deer (*Capreolus capreolus*) is exposed to various environmental variables across its range and is recognized as one of the most adaptable species among cervids due to its ability to withstand significant anthropogenic pressure and thrive in landscapes impacted by human activity. The existence of two mtDNA lineages in this species—the European and the Siberian (an introgression of *C. pygargus* mtDNA into *C. capreolus*)—was demonstrated based on an analysis of the mitochondrial control region. The Siberian lineage is the most frequent in the eastern part of the continent and declines toward Central Europe. The European lineage consists of three clades (Central, Eastern, and Western), each composed of several haplogroups.

In our study, which primarily focuses on the genetic background of roe deer ecotypes, we estimated the genetic structure of roe deer based on the whole mitochondrial genome. We employed wholegenome sequencing at 15-fold depth for 40 roe deer samples from Slovenia and Poland. Additionally, we used publicly available mitogenomes. The MitoZ tool was used for mitogenome assembly and annotation, followed by multiple sequence alignment using MAFFT and network construction. To study genetic differentiation between ecotypes, we analysed approximately 1 million unlinked nuclear singlenucleotide polymorphisms (SNPs) from the nuclear genome to construct a hierarchical clustering tree and perform multidimensional scaling (MDS) plotting. Our analysis confirmed the presence of the three mitochondrial clades of roe deer in Europe. More complex structure was revealed by analysing SNP data across the roe deer ecotypes.



<sup>&</sup>lt;sup>1</sup> University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia

# Genetic diversity of the harvest mouse (*Micromys minutus*): new data of the mtDNA cyt b and control region from central part of North Eurasia and Far East

Yalkovskaya, Lidia<sup>1\*</sup>; Pilevich, Dar'ya<sup>1</sup>; Kartavtseva, Irina<sup>2</sup>; Sheremetyeva, Irina<sup>2</sup>; Boeskorov, Gennady<sup>3</sup>; Borodin, Aleksandr<sup>1</sup>

<sup>1</sup> Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Science, Yekaterinburg, Russia

- <sup>3</sup> Institute of Diamond and Precious Metals Geology, Siberian Branch of the Russian Academy of Science, Yakutsk, Russia
- \* lida@ipae.uran.ru

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The harvest mouse (Micromys minutus P., 1771) is the smallest rodent of the family Muridae of Eurasia, whose range occupies a significant part of the forest and forest-steppe zones of the Palaearctic from northern Spain and Great Britain to the Korean Peninsula, as well as the islands of Japan and Taiwan. The species inhabits a wide variety of open habitats, including gardens and arable land, drainage ditches, and grain or rice paddies. One of the main conditions for successful M. minutus existence is the presence of habitats with high and dense herbage. For the island (Great Britain, Japan) and Central European populations, a decrease in number and a fragmentation of the range has been noted, caused by anthropogenic impact. At the same time, a number of captures of *M. minutus* beyond the eastern boundary of the distribution area of the species have been explained by the broadening of the range as a result of climate change. In this context, understanding of the processes underlying the formation of the intraspecific genetic structure, the assessment of the genetic diversity of populations and the degree of relationship between them are very important. A number of studies are devoted to the intraspecific genetic variability in *M. minutus*, phylogeny and phylogeography of the genus *Micromys*. However, there is not much data on the central part of Northern Eurasia and almost no data on the Far East. Therefore, cyt b (1140 bp) and control region (CR - 851 bp) sequences of 26 specimens of *M. minutus* from 15 localities in the East European Plain, Urals, Eastern and Western Siberia, and the Far East were analysed. For cyt b, CR and concatenated sequences of both markers 16, 15 and 19 haplotypes, respectively, have been described. Phylogeographic analysis with the inclusion of new data has revealed the existence of four new mitochondrial lineages: Central Eurasia (from the central part of the East European Plain to Altai in the southeast and Yakutia in the northeast), Eastern Siberia (Cis-Baikal Region) and two Far East linages. Three other lineages, namely Europe (from Great Britain to the northwest part of the East European Plane), Taiwan (Taiwan Island) and Japan-Korea (Japanese islands and Korean Peninsula) have been described previously. The genetic diversity and demographic analysis of *M. minutus* allowed to suggest that the modern species genetic structure and the species range have been formed during the Late Pleistocene - Holocene period under the global climate change, the dynamics of physical and geographical conditions of the regions, and, possible, anthropogenic influence.

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<sup>&</sup>lt;sup>2</sup> Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Science, Vladivostok, Russia

# The impact of hybridization between sika deer (*Cervus nippon*) and red deer (*Cervus elaphus*) on their genotype in Lithuania

Genevičienė, Evelina<sup>1</sup>; Griciuvienė, Loreta<sup>1</sup>; Ražanskė, Irma<sup>1</sup>; Lipatova, Indrė<sup>1</sup>; Aleksandravičienė, Asta<sup>1</sup>; Kibiša, Artūras<sup>1</sup>; Paulauskas, Algimantas<sup>1\*</sup>

<sup>1</sup> Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania \* algimantas.paulauskas@vdu.lt

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Hybridization between closely related deer species, such as sika deer (*Cervus nippon*) and red deer (*Cervus elaphus*), has become a growing concern due to its potential impacts on native genetic diversity. This study aims to identify the genotypes of hybrid individuals between these species in Lithuania using microsatellite markers to assess the extent and implications of hybridization. We analysed DNA samples from a representative population, employing a set of highly polymorphic microsatellite markers that target species-specific allelic variations. Preliminary results indicate that hybridization occurs between sika deer and red deer populations in Lithuania, revealing distinct hybrid genotypes. These findings highlight the importance of monitoring hybridization levels to preserve the genetic integrity of native red deer populations. Further research will expand on these results to develop strategies for managing and conserving deer populations affected by interspecies gene flow.



### Wild boar and mouflon on Elba Island: eradication or control? A multidisciplinary and integrated feasibility study as a guiding tool

Paoloni, Daniele<sup>1\*</sup>; Carlini, Eugenio<sup>1</sup>; Da Re, Riccardo<sup>2</sup>; Dell'Agnello, Filippo<sup>3</sup>; Ercolini, Riccardo<sup>1</sup>; Laghetto, Giacomo<sup>2</sup>; Lazzaro, Lorenzo<sup>3</sup>; Mastrodomenico, Daniela<sup>1</sup>; Sposimo, Paolo<sup>3</sup>; Zibordi, Filippo<sup>1</sup>; Giannini, Francesca<sup>4</sup>; Sammuri, Giampiero<sup>4</sup>

<sup>1</sup> Istituto Oikos srl – Impresa Sociale, Milano, Italy

- <sup>2</sup> ETIFOR srl società benefit, Padova, Italy
- <sup>3</sup> NEMO srl, Firenze, Italy

<sup>4</sup> Tuscan Archipelago National Park, Livorno, Italy

\* daniele.paoloni@istituto-oikos.org

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In addition to technical evaluations, wildlife management must be based on an integrated approach aimed at assessing the overall feasibility of the intervention from a scientific, regulatory, social, and economic point of view, highlighting threats. This applies even more when the species involved are mammals and when acting in anthropized contexts. Elba is the 3<sup>rd</sup> largest Italian island in terms of size and resident population and is home to two allochthonous ungulates, wild boar (*Sus scrofa*) and mouflon (*Ovis gmelini musimon*); about half of its territory is included in the Tuscan Archipelago National Park (PNAT). Wild boar and mouflon were introduced to the Island of Elba in the 1960s/70s for hunting and ornamental purposes, but today they represent a pressure factor for about half of the 25 habitats of conservation interest (Annex I Habitats Directive) on the island as well as a threat to road safety and agricultural activities on the island.

A multidisciplinary study was carried out between June 2023 and March 2024. It analysed the regulatory and socio-cultural framework and modelled the trend of the two animal populations under two different management regimes – permanent control and eradication. An estimate of economic costs was also performed. The social context was investigated through a face-to-face questionnaire administered to 268 residents and through thematic workshops addressed to local stakeholders. 77% of the interviewees from Elba considered the presence of wild boar problematic, while only 34% considered mouflon as problematic species. Citizens have shown the need to increase culling, but are wary of the 'eradication' scenario; the opposite is recorded among local stakeholders. The population models showed that to achieve eradication it is necessary to increase the culling quotas (last three years: 1078 wild boar/year and 276 mouflon/year, respectively). In the two intermediate scenarios (wild boar population of 3345 individuals and mouflon population of 1500 individuals), to achieve eradication, annual culling quotas for five years should be 1500 wild boar/year and 400 mouflon/year. The cost of eradication is estimated at €10.6 million. For comparison, over the five-year period 2019-2023, more than €1 million was spent on management activities for the two species within the protected area, without taking into account social and ecosystem costs. Analyses showed that the technically most feasible option is eradication. A strong hesitation is, however, linked to the social context. If the implementation of an effective action of involvement and participation of priority stakeholders and residents as well as tourists can foster acceptance at the local level, the overall scenario, at the national level, appears to be out of the control of the PNAT and the other institutions operating on the island.



### Chemical susceptibility of trees to grey squirrel (*Sciurus carolinensis*) bark-stripping damage

Ash, Alexandra K.<sup>1,4\*</sup>; Pickett, John<sup>2</sup>; Spake, Rebecca<sup>3</sup>; Kandil, Sahar<sup>2</sup>; Roche, Elizabeth<sup>2</sup>; Gill, Robin M.A.<sup>4</sup>; Nichols, Christopher P.<sup>5</sup>; Dutton, Charles<sup>6</sup>; Doncaster, Patrick C.<sup>1</sup>

- <sup>1</sup> University of Southampton, Faculty of Environmental and Life Sciences, School of Biological Sciences, Southampton, UK
- <sup>2</sup> Cardiff University, School of Chemistry, Cardiff, UK
- <sup>3</sup> University of Southampton, Faculty of Environmental and Life Sciences, School of Geography and Environmental Science, Southampton, UK
- <sup>4</sup> Forest Research, Centre for Forest Protection, Alice Holt Lodge, Farnham, Surrey, UK
- <sup>5</sup> Woodland Trust, Kempton Way, Grantham, Lincolnshire, UK
- <sup>6</sup> Charles Dutton & Co (Dorset) Ltd, Dorset, UK
- \* A.K.Ash@soton.ac.uk

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Invasive grey squirrels (*Sciurus carolinensis*) in Britain peel the outer bark from broadleaf trees to access the underlying phloem tissue layer, reducing timber potentials and biodiversity. Trees are highly variable in their susceptibility to squirrel damage, and while previous research has identified characteristics that relate to susceptibility in broadleaf trees, the underlying causes for variation in tree susceptibility is unknown. Plant chemistry is known to influence mammalian herbivory and foraging decisions through smell and taste. However, there are no investigations that have identified chemical factors contributing to the susceptibility or resistance of trees to squirrel damage.

To investigate the potential for tree chemistry to influence susceptibility, we identified and described the volatile organic compounds (VOCs) from the fresh foliage of ten broadleaf clones varying in their susceptibility to grey squirrel damage. VOCs were collected from the foliage via dynamic headspace sampling and were identified by gas chromatography mass spectrometry (GC-MS). Chemical compounds that varied qualitatively with clone type were identified using multivariate generalised linear models (manyqlm). The compounds identified also varied quantitatively with clone type, where quantitative differences were visualised with physical characteristics using a self-organising map (SOM) and Sankey diagram. We then explored the effects of the main identified VOCs from two clones varying in susceptibility to squirrel damage on squirrel behaviour in three Hampshire woodlands. We hypothesized differences in behavioural responses between VOCs identified from trees highly susceptible and least susceptible to squirrel damage to a mineral oil control. Generalised linear mixed effects models (GLMM) were used to model behavioural differences as a function of various VOCs with treatment and time as fixed effects, and site and feeding hopper as random factors. Squirrel behavioural responses varied with treatment, and key behavioural responses such as the time squirrels spent accessing hopper bait was significantly reduced in the presence of VOCs identified from least susceptible clones. Differences in behavioural responses to various VOCs may explain the susceptibility of trees to damage and mediate the interactions between squirrels and trees in the context of their bark-stripping behaviour.



# Assessing the presence of grey squirrel (*Sciurus carolinensis*) in Tuscany: a survey using hair tubes

Avetta, Valeria<sup>1\*</sup>; Agostini, Giulia<sup>2</sup>; Bozzi, Riccardo<sup>2</sup>; Ferretti, Marco<sup>3</sup>; Martini, Matilde<sup>2</sup>; Pucci, Chiara<sup>2</sup>; Romeo, Giorgia<sup>3</sup>; Santini, Giacomo<sup>1</sup>; Zaccaroni, Marco<sup>1</sup>

<sup>1</sup> University of Florence, Department of Biology, Florence, Italy

- <sup>2</sup> University of Florence, Department of Agriculture, Food, Environment and Forestry (DAGRI), Florence, Italy
- <sup>3</sup> Tuscany Region, Wildlife Department, Florence, Italy
- \* valeria.avetta@unifi.it

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Grey squirrel (*Sciurus carolinensis*) is a North American species that has become invasive in parts of Europe, posing a serious threat by competing with the native red squirrel (*Sciurus vulgaris*) and causing its population decline and local extinction in Great Britain, Ireland, and northern Italy.

We investigated the possibility of establishing a new population of *Sciurus carolinensis* in central Italy (Tuscany) conducting a survey in areas where the presence of the grey squirrel had been previously reported and where recent sightings were made. We sampled the study areas by using hair tubes, a non-invasive technique that has proven successful in detecting different small arboreal species, including red and grey squirrels. In one area (Arezzo), where the first sighting of grey squirrel was in 2012, we detected the presence of red squirrel which was well distributed across the study area. We can therefore exclude that a population of concern of the invasive species has established at this location, indicating that the originally introduced individual was not able to form a new growing population.



### Immunocontraceptive vaccines as fertility-control measure in the invasive raccoon

Huijsmans, Tim<sup>1\*</sup>; Schröder, Myriam<sup>2</sup>; Börner, Konstantin<sup>3</sup>

<sup>1</sup> Leibniz Institute for Zoo and Wildlife Research, Department of Reproduction Management, Berlin, Germany

<sup>2</sup> Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany

<sup>3</sup> Leibniz Institute for Zoo and Wildlife Research, Department of Ecological Dynamics, Berlin, Germany

\* huijsmans@izw-berlin.de

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In the 1930s, the first raccoons (Procyon lotor) were released into the wild in Central Europe. After several decades, raccoons have spread throughout Europe and are identified as an invasive alien species, which forms a threat to, amongst others, the European pond sliders and many bird species. Furthermore, the raccoon spreads the raccoon roundworm, a zoonosis, and causes property damages. In Germany alone, the number of raccoons has been estimated to exceed 2 million. Attempts to manage or eradicate raccoons have mainly focused on hunting and trapping. However, due to the extreme adaptive capacity of the species and relatively high reproductive rate, the population is still growing. Hence, the development of new management tools is of crucial importance. In several species, immunocontraceptives have proven to be a valuable fertility-control tool. A number of contraceptives are available, however, their efficacy, side effects, and the effect on the population dynamic have not been tested in raccoons. Two contraceptives are especially of interest: the porcine zona pellucida (pZP) vaccine and the gonadotrophin releasing hormone (GnRH) vaccine. In a positive scenario, a single vaccination could lead to lifetime infertility. The vaccine targets females, but the territorial behaviour of males remains present, therefore an influx of new raccoons in the area is prevented. In addition, the peak in the energetic demand during the gestation and lactation will not take place, therefore decreasing the negative effect of raccoons on local fauna.

In our study, two vaccines will be first tested in a captive environment, where the efficacy and side effects will be tested on 10 raccoons. The efficacy will be determined using blood samples to detect the development of antibodies over a period of 3 to 4 years. During the same period, mate attempts will be conducted by introducing potent males. Consecutive ultrasound examinations will be performed to test for pregnancy. Side effects will be tested in the first phase of the trial. At its end, raccoons will be castrated surgically to examine the reproductive tract. This will help to identify any long-term side effects of the vaccination. Given the average life expectancy of 3.1 years of raccoons in the wild, a study over 3 to 4 years will enable us to identify whether any of the methods can provide life-time infertility with minimum negative effects on health and welfare. In order to test the effect of immunocontraception in a wild population, smart traps will be developed. These traps will identify raccoons and their sex, and will have an arm that can apply the vaccination without human interference, thereby making the fertility-control cost-effective, whilst remaining species-specific. Furthermore, a population estimate will be conducted pre- and post-vaccination, to determine the effect of the fertility control method on the population dynamic. This overarching approach encompassing the effect of immunocontraceptives on the individual and population level could form an important fertility-control tool to manage the invasive raccoon. Additionally, it might serve as a model for similar approaches for other invasive species.



# Raccoon (*Procyon lotor*) as an emerging neozoon and potential reservoir for tick-borne pathogens in Germany

Maas, Lara Maria Inge<sup>1\*</sup>; Król, Nina<sup>1</sup>; Langner, Torsten<sup>2</sup>; Reinhardt, Nico<sup>3</sup>; Pfeffer, Martin<sup>1</sup>; Birka, Stefan<sup>2</sup>; Rentería-Solís, Zaida<sup>4</sup>; Sebastian, Patrick S.<sup>5</sup>; Obiegala, Anna<sup>1</sup>

<sup>1</sup> University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany

<sup>2</sup> University of Leipzig, Faculty of Veterinary Medicine, Institute of Food Hygiene, Leipzig, Germany

<sup>3</sup> LMU Munich, Department of Veterinary Sciences, Bacteriology and Mycology, Institute for Infectious Diseases and Zoonoses, Munich, Germany

<sup>4</sup> University of Leipzig, Faculty of Veterinary Medicine, Institute of Parasitology, Leipzig, Germany

<sup>5</sup> Dairy Chain Research Institute (IdICaL; CONICET-INTA), Rafaela, Argentina

\* lara\_maas@web.de

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Raccoon (*Procyon lotor*) is a medium-sized omnivore belonging to the family of small bears (Procyonidae) that originally belongs to Central and Northern America. Nowadays, it has colonized different parts of the world due to deliberate or accidental releases and is listed as invasive neozoon in Germany. The recent rise in population densities is likely to increase the risk of pathogen transmission to humans, wildlife, and domestic animals. Many zoonotic pathogens are found in raccoons worldwide, but there is a lack of epidemiological data for most of Germany's raccoon populations concerning tick-borne pathogens.

Tissue samples of 485 free-ranging raccoons obtained as hunting bag in ten federal states of Germany between 2017 and 2021 were examined for the presence of five vector-borne pathogens (*Rickettsia* spp., *Borrelia burgdorferi* sensu lato (s.l.), *Bartonella* spp., *Babesia* spp., *Neoehrlichia mikurensis*) using molecular methods. *Borrelia burgdorferi* s.l. was detected in 21 (6.3%) raccoons, identified as *Borrelia afzelii* and *Borrelia burgdorferi* s.s. Multilocus Sequence Typing (MLST) of the *Borrelia burgdorferi* s.l. positive samples was conducted for the first time in raccoons showing five known STs (24, 171, 247, 465, and 782) and four new STs (1156-1158 and 1164). *Rickettsia* spp. was found in 26 (7.8%) individuals, identified as *Rickettsia helvetica* (n = 5), *Rickettsia felis* (n = 2), and *Rickettsia conorii* subsp. *raoultii* (n = 1). *Bartonella* spp. was confirmed in 3 (0.63%) raccoons. *Neoehrlichia mikurensis* and *Babesia* spp. were not detected.

Future studies should monitor these invasive omnivore populations. However, raccoons may act as reservoir for pathogens, especially for *Anaplasma* spp. and *Rickettsia* spp., between humans, domestic animals, zoo animals, and wildlife, with a risk of infection due to their invasive behaviour and synanthropic habitat.



### Raccoon dog (*Nyctereutes procyonoides*) as a reservoir for vectorborne and zoonotic pathogens in Lithuania

Šakienė, Daiva<sup>1</sup>; Aleksandravičienė, Asta<sup>1</sup>; Griciuvienė, Loreta<sup>1</sup>; Lipatova, Indrė<sup>1</sup>; Ražanskė, Irma<sup>1</sup>; Radzijevskaja, Jana<sup>1\*</sup>; Paulauskas, Algimantas<sup>1</sup>

<sup>1</sup> Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania
\* jana.radzijevskaja@vdu.lt

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As an invasive alien species, raccoon dogs (*Nyctereutes procyonoides*) may significantly contribute to the spread of vector-borne and zoonotic pathogens due to their adaptability and broad diet. Thriving in various habitats and interacting closely with native wildlife, these animals can serve as effective reservoirs and transmitters of diseases, posing substantial risks to wild and domestic animal health. The aim of this study was to examine the presence of various pathogens in raccoon dogs and their ticks from Lithuania. Raccoon dogs and their ticks were screened via PCR using specific primers for *Babesia* spp., *Bartonella* spp., *Borrelia* spp., *Rickettsia* spp., *Anaplasma* spp., and *Francisella tularensis*. Phylogenetic analysis of the obtained sequences showed *B. microti*, *R. monacensis*, *R. helvetica*, *B. afzelii*, *B. miyamotoi*, *B. valaisiana* and *A. phagocytophilum* in the tested samples, while *F. tularensis* pathogens were not detected. Our results suggest that raccoon dogs and their ticks may play an important role for the transmission of vector-borne and zoonotic pathogens in Lithuania. However, further studies are needed to fully understand the extent of their involvement in pathogen transmission.



#### Health, zoonotic pathogens and parasites

#### SARS-CoV-2 infection in farmed minks from Lithuania

Kibiša, Paulius<sup>1</sup>; Pilevičiene, Simona<sup>2</sup>; Janeliunas, Žygimantas<sup>2</sup>; Radzijevskaja, Jana<sup>3</sup>; Paulauskas, Algimantas<sup>3\*</sup>

<sup>1</sup> Public institution Kaunas city polyclinic, Kaunas, Lithuania

<sup>2</sup> National Food and Veterinary Risk Assessment Institute, Lithuania

<sup>3</sup> Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania

\* algimantas.paulauskas@vdu.lt

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Lithuania ranks among the ten countries worldwide with established mink farming industries. In 2020, over 1.2 million minks were bred across 57 farms in Lithuania. On November 24, 2020, SARS-CoV-2 infection in minks was detected for the first time in a farm in the Jonava region. Subsequently, nationwide surveillance was conducted across all mink farms in Lithuania. Samples from dead and apparently healthy minks were collected in 15 mink farms located in 12 Lithuanian districts. Genomic sequencing of SARS-CoV-2 strains from these samples was performed to assess viral variants circulating in Lithuanian mink farms and their distribution across various geographic locations. Full-length virus genome sequencing was performed in Illumina MiSeq Sequencer. The genomes were assembled using the Illumina BaseSpace software DRAGEN COVID Lineage. The Fasta format of whole genome sequences was used for clade and lineage assignment using online tools: Nextclade and GISAD.

A total of 276 variable nucleotides were detected among the analysed 47 SARS-CoV-2 genome sequences derived from minks. Phylogenetic analyses and genotype comparisons were made to determine the relationship between mink-associated SARS-CoV-2 strains and those infecting humans. Phylogenetic analysis revealed the circulation of seven GISAID clades or four Nextstrain clades in the minks during the study period. Analysed strains belonged to ten lineages (Nextclade; GISAID). Distribution of CoV-2 variants in minks and humans in Lithuania over clades and locations and phylogenetic relationships between SARS-CoV-2 strains derived from minks in Lithuania and other European countries were analysed. The virus's evolution within an animal reservoir could result in new zoonotic sources, increasing the likelihood of recurrent transmission of emerging SARS-CoV-2 variants from minks to humans and other mammals.



#### Health, zoonotic pathogens and parasites

### Vector-borne zoonotic pathogens in domestic cats in urban areas of Lithuania

Radzijevskaja, Jana<sup>1\*</sup>; Razgūnaitė, Miglė<sup>1</sup>; Lipatova, Indrė<sup>1</sup>; Karvelienė, Birutė<sup>2</sup>; Zamokas, Gintaras<sup>2</sup>; Laukutė, Monika<sup>1,2</sup>; Paulauskas, Algimantas<sup>1</sup>

<sup>1</sup> Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania

<sup>2</sup> Lithuanian University of Health Sciences, Faculty of Veterinary Medicine, Kaunas, Lithuania

\* jana.radzijevskaja@vdu.lt

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According to the European Pet Food Industry Federation report (2022), Europe is home to approximately 127.2 million domestic cats, with 26% of households owning at least one cat, making them the most popular pet choice across the continent. In Lithuania, around 70% of households have at least one dog or cat, with 114,600 officially registered domestic cats. Despite their benefits to humans, cats can act as reservoirs or accidental hosts for various pathogens transmitted by fleas, ticks, and mosquitoes, posing transmission risks to humans and other animals.

This study aimed to investigate the prevalence and genetic diversity of vector-borne pathogens in feline populations and their ectoparasites in urban areas of Lithuania. Blood samples were collected from 543 cats (both owned and those from animal shelters) in densely populated regions of central (Kaunas city) and western (Klaipėda city) Lithuania. Ectoparasites (153 fleas and 321 ticks) were collected from owned cats in seven Lithuanian cities. Molecular methods were used for pathogen detection, complemented by morphological blood tests to identify some pathogens in blood samples. In domestic cats and their ectoparasites, we detected two causative agents of cat scratch disease, *Bartonella henselae* and *Bartonella clarridgeiae*, as well as ruminant-associated *Bartonella* sp., and haemotropic *Mycoplasma haemofelis* and '*Candidatus* M. haematominutum'. Human pathogenic *Rickettsia helvetica*, *R. conorii* subsp. *raoultii*, and *R. felis* were also identified. This study highlights the presence of diverse vector-borne pathogens in cats from densely populated regions, likely due to increased exposure to vectors in urban environments. The detection of these zoonotic pathogens in domestic cats, both pets and those in shelters, represents a significant public health concern in urban areas, underscoring the need for regular screening and effective vector control initiatives.



#### Health, zoonotic pathogens and parasites

### Detection of *Toxoplasma gondii* antibodies in slaughtered cattle and hunted red deer in Lithuania

Laukutė, Monika<sup>1,2\*</sup>; Karvelienė, Birutė<sup>2</sup>; Zamokas, Gintaras<sup>2</sup>; Stadalienė, Inga<sup>2</sup>; Paulauskas, Algimantas<sup>1</sup>; Radzijevskaja, Jana<sup>1</sup>

<sup>1</sup> Vytautas Magnus University, Research Institute of Natural and Technological Sciences, Kaunas, Lithuania

<sup>2</sup> Lithuanian University of Health Sciences, Faculty of Veterinary Medicine, Kaunas, Lithuania

\* monika.laukute@lsmu.lt

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Toxoplasmosis is one of the most common human zoonoses caused by undercooked meat. *Toxoplasma gondii* is a foodborne parasitic protozoan that poses significant concerns for public health. The disease is estimated to affect around one-third of the world's human population and can affect almost all warmblooded animals. Detecting antibodies against *T. gondii* in livestock, such as cattle, and wildlife, such as deer, is crucial for understanding the spread and epidemiology of the parasite. The aim of the study was to analyse the prevalence of *T. gondii* in cattle housed under different conditions in Lithuania, as well as in wild red deer, and to assess the potential contamination of meat.

Beef diaphragm muscle samples were collected from three slaughterhouses during the period from January to April 2024. Based on their housing systems, the cattle were classified as kept in closed and open environments. Deer diaphragm samples were collected during the hunting season in different regions of Lithuania from November 2022 to January 2023. The detection of T. gondii antibodies in meat juices was conducted using an indirect ELISA method (ID Screen® Toxoplasmosis Indirect Multispecies, ID.vet, France). A total of 47 beef diaphragm and 68 venison diaphragm samples were analysed. The findings revealed that 15 beef samples tested seropositive, indicating a prevalence rate of 31.9%. Additionally, seven samples were classified as equivocal (14.9%), while 25 were confirmed negative (53.2%). The average age of the sampled cattle was found to be 36.5 months. Notably, 12.8% of the cattle were housed in closed systems, whereas 87.2% were in open systems. Importantly, all positive samples originated from cattle in open systems, with a mean age of 39.2 months. Furthermore, no infections were identified in cattle younger than 12 months. Our findings showed a significantly higher prevalence of *T. gondii* infection in cattle housed in open systems than those in closed systems (P<0.05). The testing of venison samples showed that 12 of them were seropositive, indicating a prevalence rate of 8.2%. Additionally, four samples were classified as equivocal (2.7%), while 52 were confirmed negative (89.1%). All seropositive samples were derived from adult deer.

The results show that cattle are frequently exposed to *T. gondii*, especially when kept in open systems. In contrast, the prevalence of *T. gondii* in deer samples was considerably lower, indicating that while deer can harbour the parasite, the transmission risk from venison appears to be less pronounced compared to beef. Of particular concern is that meat is not routinely tested for this parasite, neither in animals raised in Lithuania nor in imported meat products. To ensure food safety and quality throughout the food chain, further research is essential to determine the exact prevalence of *T. gondii* and identify effective measures to minimize the potential spread of the parasite along the epizootic chain, reducing the associated risk factors for infection.



# Presence of bacteria in red deer antlers during different stages of development

Škvorc, Nikolina<sup>1\*</sup>; Bujanić, Miljenko<sup>1</sup>; Štritof, Zrinka<sup>1</sup>; Kužir, Snježana<sup>1</sup>; Konjević, Dean<sup>1</sup>

<sup>1</sup> University of Zagreb, Faculty of Veterinary Medicine, Zagreb, Croatia

\* nskvorc@vef.unizg.hr

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Red deer antlers grow and mineralize while being covered with velvet. Following the velvet shedding, hard antlers are revealed. There are still debates whether hard antlers are a dead bone or they survive for longer periods after velvet shedding. Since reddish liquid is frequently present on the cut surface of hard antlers and is by some authors described as blood, the aim of this study was to analyse presence of blood cells and bacteria in it.

Antlers from a red deer spiker (yearlings, i.e. in second year of life) were sampled in different stages of development: in the velvet (A1), two months after velvet shedding (A2), and three months after velvet shedding (A3). Antlers in the velvet were sampled in complete chemical immobilization. Hard antlers were removed with a fine-toothed saw approximately 5 cm above the pedicle-antler junction. After delivery to the Faculty of Veterinary Medicine, the sampled antler beams were divided into three segments (1 – proximal, 2 – middle, 3 – distal). Each segment was additionally sampled longitudinally and transversely. Before cutting, the surface of the antler and diamond separator were treated with 70% ethyl alcohol. The incision site was cooled with saline solution. Study was two folded, presence of bacteria was observed using microbiological analyses and using histological analysis. Swabs were taken from the cut surface and sent for bacteriological examination. The species were identified by the MALDI TOF method. The fragments were fixed in 10% neutral buffered formalin. The antler fragments were demineralised using Osteosens® solution (EDTA based) and subsequently washed in phosphate buffer. The fixed and demineralised fragments were embedded in paraffin and sliced to a thickness of 6 µm. The sections were stained with hematoxylin-eosin (HE) and BioGram Histo kit for differentiation between Gram-positive and Gram-negative bacteria in histology sections. Smears of liquid content from the antlers were stained with May-Grünwald Giemsa.

Red blood corpuscles and leukocytes were present only in A1. With BioGram Histo kit bacteria were visible only in the A2 and A3. Swabs from A2 were positive to *Erysipelothrix rhusiopathiae, Kurthia zopfii, Bacillus licheniformis, Macrococcus canis,* and *Citrobacter freundii*. Swabs from A3 were positive to *Pseudomonas flavescens*. Obligate anaerobes were not isolated from A1, A2, and A3 swabs. Study showed that antlers in the velvet contain blood without bacteria, which was expected for live tissue. After the velvet shedding, we did not find blood cells, and the analysed liquid contained bacteria, confirming the cessation of circulation during mineralization. The remaining liquid is insufficient to nourish bone cells and provides an ideal medium for bacterial growth. Most of the mentioned bacteria are present in the environment. All isolated bacteria have zoonotic potential, and infections caused by them have been confirmed in humans.



# Wild boar (*Sus scrofa*) and *Mammaliicoccus sciuri* in One Health research

Nagy, Eszter<sup>1\*</sup>; Tari, Tamás<sup>1</sup>

<sup>1</sup> University of Sopron, Institute of Wildlife Biology and Wildlife Management, Sopron, Hungary

\* nagy.gesztenye07@gmail.com

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*Mammaliicoccus sciuri* (syn.: *Staphylococcus sciuri*) is a ubiquitous bacterium among the oldest species close to the *Staphylococcus* genus. Currently, researchers show a great interest in this ancient bacterial species because of the frequent multidrug resistance feature. Since *M. sciuri* evolved before its close relatives, the staphylococci, scientists hypothesise that most of the resistance genes characteristic for the *Staphylococcus* genus evolved in this species. Most research findings support this hypothesis, since the most known antimicrobial resistance, the methicillin resistance can often be detected in *M. sciuri*. Wild boar is frequently considered as an indicator of the health of the ecosystem. Their rooting and scavenging activity support the pathogens to contaminate the animals body surface or infect their mucous membranes. Based on these facts, we hypothesised that nasal swab samples from wild boar and water samples of wild boar shallows are good sources of ubiquitously distributed bacterial species, such as *M. sciuri*. Our goal was to isolate *M. sciuri* strains from these samples and determine the antimicrobial susceptibility of these isolates. For this reason, we collected swab samples from the nasal cavity of hunted wild boar and water and mud samples from shallow pools used by the species. This sample collection was carried out in a nature conservation area to avoid areas with remarkable direct human impact.

Preliminary results supported our hypothesis, as more than fifth of the wild boar samples carried *M. sciuri*, while almost half of the shallow water samples also contained *M. sciuri*. All detected *M. sciuri* strains proved to be multidrug resistant, since they showed resistance to at least three classes of antimicrobials. The antibiotics to which the most bacterial isolates were resistant were benzylpenicillin, clindamycin, and fusidic acid. Unfortunately, we also detected *M. sciuri* isolates with cefoxitin and oxacillin resistance, which is considered as an indicator of methicillin resistance. These strains could be isolated from shallow water but not from nasal swab specimens. Molecular comparison of the two types of samples is in progress.

As a conclusion of our study, we can ascertain that wild boar is a good target to assess antimicrobial resistance in natural habitats. The bacterial species *M. sciuri* also proved to be a promising screening tool for evaluation of ecosystem health, whereas it can be isolated easily, in high prevalence, and it possesses more phenotypic characteristics of antimicrobial resistance. We believe that a bacterial surveillance in wild boar to detect *M. sciuri* and its antimicrobial susceptibility can be a good tool to estimate the influence of human activity on ecosystem health.



## Lymphocytic choriomeningitis virus: a neglected zoonotic pathogen in Germany

Mehl, Calvin<sup>1,2</sup>; Wylezich, Claudia<sup>1</sup>; Adeyemi, Olayide Abraham<sup>1</sup>; Möhrer, Fiona Joana<sup>1</sup>; Kerber, Romy<sup>3</sup>; Cadar, Daniel<sup>3</sup>; Oestereich, Lisa<sup>2,3</sup>; de Cock, Marieke P.<sup>4</sup>; Maas, Miriam<sup>4</sup>; Geiger, Christina<sup>5</sup>; Schauerte, Nicole<sup>5</sup>; Mätz-Rensing, Kerstin<sup>6</sup>; Nesseler, Anne<sup>7</sup>; Schmidt, Katja<sup>8</sup>; Höper, Dirk<sup>1</sup>; Linnenbrink, Miriam<sup>9</sup>; Beer, Martin<sup>1</sup>; Heckel, Gerald<sup>10</sup>; Ulrich, Rainer G.<sup>1,2\*</sup>

- <sup>1</sup> Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany
- <sup>2</sup> German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Germany
- <sup>3</sup> Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
- <sup>4</sup> National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Utrecht, the Netherlands
- <sup>5</sup> Zoo Frankfurt, Frankfurt am Main, Germany
- <sup>6</sup> German Primate Center, Leibniz Institute for Primate Research GmbH, Göttingen, Germany
- <sup>7</sup> Landeslabor Hessen, Gießen, Germany
- <sup>8</sup> German Cancer Research Center, Microbiological Diagnostics, Heidelberg, Germany
- <sup>9</sup> Max-Planck-Institute for Evolutionary Biology, Plön, Germany
- <sup>10</sup> University of Bern, Institute of Ecology and Evolution, Bern, Switzerland

\* calvin.mehl@fli.de

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Lymphocytic choriomeningitis virus (LCMV) is a zoonotic arenavirus transmitted by house mice (Mus musculus) through contact with infectious secreta and excreta. Vertical transmission in house mice can result in death of the embryo, while surviving offspring will be persistently infected and shed the virus throughout their lives. Human infections may present with flu-like symptoms, but in severe cases meningitis and encephalitis may develop. LCMV infection poses a significant threat to organ transplant patients, because they are immunosuppressed. In pregnant women, LCMV infection may have significant teratogenic effects, often presenting with deformities of the brain and eyes of the foetus. LCMV was also identified as the etiological agent responsible for callitrichid hepatitis (CH), a lethal infection in New World primates. Despite these, LCMV is not a notifiable disease in Germany and little is known about the virus' prevalence and diversity. LCMV and reactive antibodies were detected in humans and mice from Germany in the 1960's, with the highest prevalence in North Rhine-Westphalia. This was also the same region in which the first German CH outbreak was reported in 2001. LCMV was detected in nine Emperor marmosets from another zoological garden in Germany in 2014. Continued assessment of LCMV in this zoo revealed an increasing viral prevalence in wild house mice between 2021 and 2023, as well as two further CH cases. High-throughput sequencing and phylogenetic analyses showed that two distinct lineages (I and II) of LCMV were present in the zoo, though only lineage II was detected in primates. The continued co-occurrence of these different strains (lineages I and II) resulted in coinfections and possible reassortants in wild house mice. This may have significant implications for virus evolution, the emergence of novel strains and viral epidemiology. LCMV screening was expanded to other regions of Germany and several other European countries (2005-2023). This resulted in the discovery of a novel lineage of LCMV in wood mice (Apodemus sylvaticus) from the south of Germany. Despite screening more than 2,100 small mammals, only a single positive house mouse was detected in the Netherlands. These findings suggest that LCMV is maintained in nature primarily through patches of high prevalence, and additional small mammal species may be hosts to yet undiscovered arenaviruses.



# The occurrence of Seoul orthohantavirus in wild and captive rats in Europe

Mehl, Calvin<sup>1,2</sup>; Drewes, Stephan<sup>1</sup>; Heuser, Elisa<sup>1</sup>; Trimpert, Jakob<sup>3</sup>; Kunec, Dusan<sup>3</sup>; de Cock, Marieke P. <sup>4</sup>; de Vries, Ankje<sup>4</sup>; Maas, Miriam<sup>4</sup>; Krüger, Detlev H.<sup>5</sup>; Latz, Andreas<sup>6</sup>; Hofmann, Jörg<sup>5</sup>; Dreesman, Johannes<sup>7</sup>; Heckel, Gerald<sup>8</sup>; Ulrich, Rainer G.<sup>1,2\*</sup>

- <sup>1</sup> Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany
- <sup>2</sup> German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Germany
- <sup>3</sup> Freie Universität Berlin, Institute of Virology, Department of Veterinary Medicine, Berlin, Germany
- <sup>4</sup> National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Bilthoven, Utrecht, the Netherlands
- <sup>5</sup> Charité–University Medicine Berlin, Institute of Virology, Berlin, Germany
- <sup>6</sup> NovaTec Immundiagnostica GmbH, Veterinary Department, Dietzenbach, Germany
- <sup>7</sup> Public Health Agency of Lower Saxony, Department of Infectious Diseases, Hannover, Germany
- <sup>8</sup> University of Bern, Institute of Ecology and Evolution, Bern, Switzerland
- \* rainer.ulrich@fli.de

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Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) are synanthropic rodents, and reservoirs for a cocktail of zoonotic pathogens, including Seoul orthohantavirus (SEOV). First described in Korea, SEOV is now known to have a global distribution. In humans, SEOV infection can cause hemorrhagic fever with renal syndrome (HFRS), with a case fatality rate of more than 1%. The virus can be transmitted through inhalation of aerosolized particles or through bites. Although parts of Asia have reported high SEOV prevalence in wild rats, with human infections being common, only a few studies in the Netherlands, UK and Belgium have reported SEOV detections.

Here, we aimed to assess the current status of SEOV in Germany and the Netherlands by screening wild and captive rats for SEOV RNA and reactive antibodies. More than 1,500 wild, pet, lab and breeder rats sampled from 2013-2021 were screened. Although reactive antibodies were detected in seven individuals, no RNA was detected in any of these animals. However, we identified three human SEOV infection clusters in Germany (2019-2021), with patients being admitted to intensive care. In all three outbreaks, the patients had recently purchased pet fancy rats. Investigation of these rats, and rats from the breeders who had sold these rats, revealed SEOV RNA and reactive antibodies at high prevalence. Full genome sequences (S, M and L segments) revealed nearly identical sequences between the patients and their pet rats. Virus sequences from all three outbreaks clustered closely together, suggesting a common source of infection. These sequences were also very similar to those found in rats and patients in other European countries. Together, these findings suggest that SEOV is rare in wild rats from Europe, but is present in private breeding facilities. The trade of fancy rats in Europe likely facilitates the spread of SEOV and outbreaks in pet owners in Europe. Although we found little evidence of SEOV in wild rats, spill-over from captive breeding may allow the virus to enter native populations, while pet trade may facilitate the spread to new areas.



## The presence of tularemia (*Francisella tularensis*) in *Apodemus* sp. in the Belgrade forest area

Radovanović, Tamara<sup>1</sup>; Jokić, Goran<sup>2\*</sup>; Blažić, Tanja<sup>2</sup>; Veinović, Gorana<sup>3</sup>; Radojičić, Sonja<sup>1</sup>; Stević, Nataša<sup>1</sup>

<sup>1</sup> University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia

<sup>2</sup> Institute of Pesticides and Environmental Protection, Belgrade, Serbia

<sup>3</sup> University of Belgrade, Institute for Medical Research, Belgrade, Serbia

\* jokicg@ptt.rs

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Tularemia, zoonosis caused by *Francisella tularensis*, is considered endemic to the Balkan Peninsula. Main reservoirs of the disease are rodents, which usually show no clinical signs and are a potential disease source for other animals and humans. We investigated the presence of *Francisella tularensis* in *Apodemus* sp. The capture of individuals of field rodents was carried out at the edge of the forest, near the promenade or places for recreation in the Belgrade area. A total of 96 small field rodents were collected during the autumn 2023 in three forested regions. Tissue samples were taken and pooled samples were prepared for all tested animals (71 *A. agrarius*, 13 *A. flavicolis*, 12 *A. sylvaticus*). *F. tularensis* was detected by PCR, using primers TUL4-435 and TUL4-863. 13 samples tested positive on PCR while 7 of those have been proven to belong to *F. tularensis* subsp. *holartica* by sequencing. *F. tularensis* was detected in animals in all three areas. All positive individuals belong to *A. agrarius*. It was concluded that *F. tularensis* is present in wood mice in the Belgrade region.

The present study was funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia (Grants No. 451-03-66/2024-03/200143 and 451-03-66/2024-03/200214).



## Wild rodents as carriers of leptospirosis in areas of increased human presence

Tošić, Božana<sup>1\*</sup>; Savić, Sara<sup>2</sup>; Žekić, Marina<sup>2</sup>; Miljević, Milan<sup>3</sup>; Čabrilo, Borislav<sup>1</sup>; Bjelić-Čabrilo, Olivera<sup>1</sup>

<sup>1</sup> University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia

<sup>2</sup> Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia

<sup>3</sup> University of Belgrade, Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, Department of Genetic Research, Belgrade, Serbia

\* bozana.tosic@dbe.uns.ac.rs

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From August 2019 to June 2020, research was conducted on the role of rodents as natural reservoirs in the spread of zoonoses in eight selected localities in the territory of Vojvodina Province, Serbia. The rodent sample included 134 individuals: Apodemus sylvaticus (31 individuals), A. agrarius (37), A. flavicollis (62), Micromys minutus (1), Mus musculus (1), Microtus arvalis (1), and M. agrestis (1). As part of the study, organ samples (heart and kidneys) of these species were tested for the presence of Leptospira sp. bacteria. All samples were tested using real-time PCR method on the Genesig q16 device, following the manufacturer's standard protocol with "Primerdesign" kits. The detection of Leptospira sp. was conducted through the following procedures: (i) isolation-extraction of DNA from samples using the commercial kit "Genesig easy DNA/RNA Extraction kit", and (ii) real-time PCR reaction using the commercial kit "Leptospirosis Genesig easy kit", manufactured by "Primer design". The presence of Leptospira sp. was confirmed in 12 individuals, namely 11 individuals of striped field mouse (A. agrarius) from three lowland localities close to aquatic habitats, and in one yellow-necked mouse (A. flavicollis) from an area of higher elevation. Leptospira sp. bacteria were detected in the kidneys, which are the preferred site for these bacteria in all animals and humans. The obtained findings indicate the existence of critical endemic areas for Leptospira sp. in the territory of Vojvodina Province, where the occurrence of these diseases in humans is registered every year. The results are highly valuable from an epidemiological point of view for the improvement of public health and are very important in the context of applying the One Health approach to the problem of zoonotic diseases whose reservoirs are mouse-like rodents.

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## RodentGate: future rodent management for pig and poultry health

Leirs, Herwig<sup>1</sup>; Vanden Broecke, Bram<sup>1,2</sup>; Sluydts, Vincent<sup>1</sup>; Belmain, Steven R.<sup>3</sup>; Meerburg, Bastiaan G.<sup>4</sup>; Jacob, Jens<sup>5</sup>; Szczotka-Bochniarz, Anna<sup>6</sup>; other members of the RodentGate team

<sup>1</sup> University of Antwerp, Department of Biology, Evolutionary Ecology Group, Antwerpen, Belgium

- <sup>2</sup> University of Ghent, EcoBird, Belgium
- <sup>3</sup> University of Greenwich, Natural Resources Institute, UK
- <sup>4</sup> Dutch Pest & Wildlife Expertise Centre, Wageningen, the Netherlands
- <sup>5</sup> Julius Kühn-Institut, Munster, Germany
- <sup>6</sup> National Veterinary Research Institute, Department of Swine Diseases, Pulawy, Poland
- \* Herwig.leirs@uantwerpen.be

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Apart from consuming and spoiling animal feed, and damaging infrastructure in and around buildings, rodents are a considerable threat to animal and human health. They can cause direct stress in pigs and poultry but are mainly important as carriers of pathogens. They can pick up the infection from infected pigs or poultry and spread it within and between farms, act as a bridge between wild fauna and livestock, and maintain the infection locally when a farm is emptied and decontaminated after a disease outbreak or livestock turnover. An important approach for on-farm rodent control is the use of rodenticides but new restrictions on their use pose new challenges for efficient rodent management on farms.

The project RodentGate investigates rodent-related risks for animal health in the pig and poultry industry and how this might change with altered rodent control. For two years, the RodentGate consortium has trapped more than 650 rodents on pig and poultry farms in different parts of the UK, Belgium, the Netherlands, Germany, and Poland. Molecular analysis using qPCR has revealed that these rodents are harbouring various pathogens which are known to infect poultry and pigs, such as *Brachyspira* sp., pathogenic *Leptospira* species, *Salmonella* and *Lawsonia intracellularis*. Besides showing that these rodents harbour different infections, the consortium was also able to determine the rodents' individual movement and social behaviour, which were further used in epidemiological models. In addition to uncovering infections, the consortium has tracked the movements and social behaviours of individual rodents, which were then integrated into epidemiological models. These models allowed us to assess the effectiveness of different rodent management strategies on the transmission rates of different pathogens. Our findings suggest that merely reducing rodent populations through lethal means is not sustainable, as it may lead to a higher prevalence of disease in the long term. Instead, the most effective long-term strategy is to lower the carrying capacity for rodents on farms by limiting their access to food and shelter.



## Rodenticide resistance and environmental monitoring

## **Environmental impact of anticoagulant rodenticides**

Regnery, Julia<sup>1\*</sup>; Friesen, Anton<sup>2</sup>

<sup>1</sup> Federal Institute of Hydrology, Department Biochemistry, Ecotoxicology, Koblenz, Germany

<sup>2</sup> German Environment Agency, Section IV 1.2 Biocides, Dessau-Rosslau, Germany

\* regnery@bafg.de

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Exposure of wildlife to anticoagulant rodenticides (ARs) has been extensively monitored worldwide for a variety of terrestrial species that are directly or indirectly linked to rodents via the terrestrial food web. Recently, our research demonstrated the relevance of aquatic exposure pathways due to AR emissions to the aquatic environment in conjunction with urban rat management practices such as sewer baiting and bait application in close proximity of surface water bodies. In Germany, residues of second-generation ARs have been frequently detected in fish and fish-eating predators from locations influenced by chemical rodent control measures, in spite of strict regulations regarding the sale, supply, and use of ARs to mitigate exposure risks. Moreover, new research data confirmed that there will be adverse effects of chronic second-generation AR exposure on fish health at concentrations relevant for surface water bodies. Unfortunately, the release of rodenticides to the aquatic environment due to the deployment of unprotected bait by wire during sewer baiting continues to be a serious environmental issue based on German survey results from 2022. Given the increasing number of technical solutions available on the market that comply with best practice guidelines, there is an urgent need to rethink (former) rat management practices in urban and peri-urban settings to prevent such AR emissions, and the ecotoxicological consequences thereof, in the future.



### Rodenticide resistance and environmental monitoring

## Identified locations for digital sensors as a warning system for resistant rat infestations on farms (digiWRaP)

Anderle, Brit<sup>1,3\*</sup>; Buchholz, Sascha<sup>3</sup>; Klemann, Nicole<sup>4</sup>; Gabriel, Doreen<sup>2</sup>; Esther, Alexandra<sup>1</sup>

<sup>1</sup> Julius-Kühn-Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Urban Green, Working Group for Non-rodent Animals and Rodenticide Resistance, Braunschweig, Germany

<sup>2</sup> Julius-Kühn-Institut (JKI) - Federal Research Centre for Cultivated Plants, Institute for Crop and Soil Science, Braunschweig, Germany

<sup>3</sup> University of Münster, Institute for Landscape Ecology, Working Group for Animal Ecology, Münster, Germany

<sup>4</sup> Consultant for rodent management and research, Warendorf, Germany

\* brit.anderle@julius-kuehn.de

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Control measures using anticoagulant rodenticides (clotting inhibitors) against resistant Norway rats (Rattus norvegicus) on agricultural farms usually begin only once rat populations are detected randomly because of numerous established individuals, posing a significant risk of pathogen infection to livestock and humans. Hygiene measures can delay a re-establishment for approximately 85 days and can contribute to successful rat control. However, the immigration of new individuals and the establishment of new populations on farms cannot be fully prevented. The aim of this project is to develop a digital warning system that already detects individual rats at a farm at an early stage and reports this to a mobile device. Farmers can then take immediate on-site action to prevent establishment of a rat population. Especially in regions with rodenticide resistance (e.g. Münsterland, North Rhine-Westphalia, Germany), this would reduce the use of the most toxic, persistent, and bioaccumulative rodenticides. This could lower the risk to non-target species and the environment as well as the infection risk. To ensure the successful use of the warning system, bait stations must be placed where rat infestations are most likely to occur. In this project, following successful control measures, the immigration and infestation by Norway rats were monitored at five different farms in the Münsterland, North Rhine-Westphalia. Activity at a total of 144 bait stations was monitored using wildlife cameras and by checking sand plates in the bait stations for tracks. Observation was concluded once a new infestation was detected, defined as activity in 20% of the bait stations during a certain time. Through statistical analyses, we determined which locations and structures are particularly attractive to rats and are thus likely to be infested first, e.g. the immediate proximity to feed stores. Based on the findings, recommendations for the optimal positioning of sensors will be developed as a guide to enable successful use of the warning system on agricultural farms.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany. The Federal Office for Agriculture and Food (BLE) provides coordinating support for future farms and future regions as a funding organisation, grant number 28DE209X21.



### Rodenticide resistance and environmental monitoring

### No evidence of anticoagulant resistance in Barcelona's rats

Ruiz-López, María José<sup>1,3\*</sup>; Franco, Sandra<sup>2</sup>; Martínez-de la Puente, Josué<sup>1,3</sup>; Ferraguti, Martina<sup>1,3</sup>; Miccolis, Emanuele<sup>5,6</sup>; Petit, Robert<sup>2</sup>; Barahona, Laura<sup>2,6</sup>; Figuerola, Jordi<sup>1,3</sup>; Montalvo, Tomas<sup>2,3,6</sup>

<sup>1</sup> Estación Biológica de Doñana (EBD), CSIC, Avda, Seville, Spain

<sup>2</sup> Agència de Salut Pública de Barcelona (ASPB), Barcelona, Spain

<sup>3</sup> CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

<sup>4</sup> Brighton Third Sector Training LTD, Brighton, UK

<sup>5</sup> Third Sector International (3Si), Sevilla, Spain

<sup>6</sup> Institut de Recerca Sant Pau (IR SANT PAU), Barcelona, Spain

\* mjruiz@ebd.csic.es

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Urban rodents, such as brown rat (*Rattus norvegicus*) and black rat (*Rattus rattus*), cause significant problems in cities and are the primary target of control efforts. Managing these synanthropic species remains a major challenge. Today, anticoagulant rodenticides are widely used worldwide to control rodent populations, including urban populations. However, despite their effectiveness, their use is sometimes compromised by the emergence of resistance. One of the best studied resistance mechanisms involves the enzyme vitamin K epoxide reductase (VKOR), the target of anticoagulant rodenticides. When anticoagulants inhibit VKOR, the resulting lack of bioavailable vitamin K disrupts the gamma-carboxylated of clotting factors, thereby affecting the coagulation. This leads to internal bleeding and, ultimately, death. Mutations in the Vkorc1 gene have been shown to confer resistance to anticoagulants in rats.

This study investigates the frequency of anticoagulant resistance genotypes in the Vkorc1 gene in rats from the Barcelona area. Brown rats undergoing rodenticide control programmes with alternating active ingredients were sampled from the sewage system between two periods: December 2016 to November 2017, when difenacoum and brodifacoum were used, and August 2021 to July 2022, when bromadiolone was used. Black rats, newly detected in Barcelona, were only sampled in the latter period as part of green space management. Exon 3 of the Vkorc1 gene was sequenced in both rat species, and Exon 1 was also analysed in black rats. Synonymous mutations, which do not lead to amino acid changes, were detected and showed no evidence of anticoagulant resistance. This suggests that the current approach in Barcelona, which uses the rotation of rodenticides and live traps as part of a broader surveillance and control strategy, has been effective in preventing the development of resistance. Future surveillance and control plans should consider diversifying methods to further improve rodent management. Implementing well-regulated control strategies and fostering community involvement in rodent management are key to maintaining rodenticide efficacy and minimising the risk of resistance.



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