

# 11<sup>th</sup> International Conference on Behaviour, Physiology and Genetics of Wildlife

October 4<sup>th</sup> - 7<sup>th</sup>, 2017  
Berlin Germany



Edited by  
Jewgenow K, Braun B, Prügel J, Lenz S, Hofer H



Contributions to the

**11<sup>th</sup> International Conference on Behaviour,  
Physiology and Genetics of Wildlife**

October 4<sup>th</sup> – 7<sup>th</sup>, 2017, Berlin, Germany

Edited by

Katarina Jewgenow, Beate Braun, Josepha Prügel, Stefanie Lenz, Heribert Hofer

Organised by

Leibniz Institute for Zoo and Wildlife Research (IZW)  
Alfred-Kowalke-Straße 17  
10315 Berlin  
Germany

[www.leibniz-izw.de](http://www.leibniz-izw.de)

European Association of Zoos and Aquaria (EAZA)  
Executive Office  
PO Box 20164  
1000 HD Amsterdam  
The Netherlands

[www.eaza.net](http://www.eaza.net)



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ISSN 1865-4436

Published by  
Leibniz Institute for Zoo and Wildlife Research (IZW)  
Alfred-Kowalke-Str. 17, 10315 Berlin (Friedrichsfelde)  
PO Box 700430, 10324 Berlin, Germany

Supported by  
Deutsche Forschungsgemeinschaft (DFG) [German Research Foundation]  
Kennedyallee 40, 53175 Bonn, Germany



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Processing of abstracts:	Josepha Prügel
Setting and layout:	Josepha Prügel, Stefanie Lenz
Cover photo:	Wolf ( <i>Canis lupus</i> ), © Heiko Anders; <a href="http://www.andersfotografiert.com">www.andersfotografiert.com</a>
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## Foreword

This volume contains the contributions to the “11<sup>th</sup> International Conference on Behaviour, Physiology and Genetics of Wildlife”, a conference jointly organised by the Leibniz Institute for Zoo and Wildlife Research (IZW) Berlin and the European Association of Zoos and Aquaria (EAZA). This series of meetings brings together specialists from different disciplines and covers a variety of topics such as research on ecology, behaviour, stress and disturbance, reproduction biology, wildlife conservation and conservation genetics. The conference has developed a tradition of fostering an exchange of ideas and methods between scientists working with free-ranging and captive animals. A particular aim of the conference is to introduce participants to new concepts, methods and techniques developed within one discipline that may be very useful to answer questions in another.

The first section of this volume contains the abstracts of invited plenary speakers, followed by summaries of the parallel sessions and the workshops and the abstracts of introductory talks to parallel sessions and workshops where appropriate. The main section contains the abstracts of submitted contributions. Contributions were invited for the following topics:

- Behavioural ecology
- Conservation biology
- Conservation genetics
- Cryopreservation of gametes and embryos for conservation breeding
- Ecophysiology of wildlife
- Reproduction management of zoo collections
- Stress and disturbance
- Urban wildlife ecology
- Wildlife detection dogs

The abstracts in this volume are organised alphabetically by author name and were published as submitted. To help you to find abstracts of interest we have included an index of first authors, a taxonomic index and an index of keywords at the end of the volume.

We thank Anne Berger, Steffen Berthold, Dagmar Boras, Marion L East, Jörns Fickel, Luca Flauaus, Daniel W Förster, Oliver P Höner, Stephanie Kramer-Schadt, Sven Kühlmann, Stefanie Lenz, Gabriele Liebich, Evangelia Layla Mpinou, Karin Müller, Sylvia Ortmann, Zjef Pereboom, Wolfgang Richter, Kathleen Röllig, Thomas Sanne, Utpala Sharma, Paul Sobtzick, Jan Vieth, Bettina Wachter and Alexandra Weyrich for their help in the preparation of the conference and its scientific programme. We are very grateful to the organisers of the workshop and parallel sessions as well as to all the colleagues who have kindly reviewed all submitted contributions.

Berlin, September 2017

Beate Braun, Katarina Jewgenow, Stefanie Lenz, Josepha Prügel, Steven Seet and Heribert Hofer

## PLENARIES

### **The evolving role of reproductive biology in zoos**

ASA CHERYL

AZA Reproductive Management Center, Saint Louis Zoo, 1 Government Drive, St. Louis, Missouri 63110, USA; asa@stlzoo.org

Reproduction has been important in zoo collections for many reasons, originally and perhaps most fundamentally to maintain or increase captive populations while avoiding importation of animals from the wild. Successful reproduction has also served as a minimal indicator of animal welfare. Concern for genetic health of captive populations led to the development of computer software to analyse genetic relationships and to generate recommendations for optimal pairings, which relied on cooperative programs among zoos, specifically Species Survival Plans (SSP) in the U.S. and European Endangered Species Programs (EEP) in Europe. A challenge for zoo managers has been that some species are difficult to breed while others reproduce prolifically. In response to the production of surplus animals, the U.S. zoo community (Association for Zoos and Aquariums: AZA) approved creation of a Contraception Advisory Group and later the AZA Wildlife Contraception Center to provide evidence-based information for limiting reproduction. More recently, a sister organization for EAZA (European Association for Zoos and Aquaria) was begun: the European Group for Zoo Animal Contraception (EGZAC). However, influenced by regional cultural differences, euthanasia has been the preferred option for some zoos or for some taxa. In contrast to species that bred prolifically, others proved more difficult. In particular, some genetically valuable individuals consistently failed to breed, which encouraged zoos to establish research programs headed by reproductive physiologists focused on assisted reproduction techniques (ART). However, the promise of ART was not fulfilled for most species, given the sometimes profound species differences in reproductive systems and responses to manipulation. A recent, growing awareness of the magnitude of reproductive failure in zoo breeding programs has raised an alarm about their unsustainability. The major reasons for these failures identified to date are mate incompatibility and female infertility due in large part of long periods without reproducing, termed „Use it or lose it“. These challenges present new opportunities for behaviourists and reproductive physiologists to contribute to animal conservation breeding programs. Recognizing the importance of these disciplines in addressing current problems, AZA expanded the role of the Contraception Center, which is now the AZA Reproductive Management Center. The RMC oversees a wide range of committees and programs with the goal of improving the sustainability of animal populations in AZA institutions.

## Ageing, stress and evolution

KIRKWOOD THOMAS

Newcastle University Institute for Ageing, Campus for Ageing and Vitality, Newcastle upon Tyne NE4 5PL, UK; tom.kirkwood@ncl.ac.uk

Evolutionary logic makes clear that the primary cause of intrinsic ageing is to be found in the limited priority that is placed by natural selection in preventing the ultimate deterioration of cells and organs that occurs through accumulation of molecular damage. A corollary to this conclusion is that species-specific patterns of ageing and longevity are also the products of how natural selection acts within the ecological context to optimise the balance between investing in growth, reproduction and maintenance, subject always to physiological and anatomical constraints. A direct consequence of this logic is that multiple forms of damage are expected to contribute to the underlying pathobiology of age-related chronic diseases and frailty. Recognising the complexity of how diverse forms of damage interact one with another, and how the responses to damage may themselves contribute to downstream deleterious effects, has enabled significant progress. Energetics and metabolism play central roles because (i) some of the damage arises through by-products of essential metabolic functions, (ii) energy is necessary to support proofreading and turnover processes such as autophagy, which when compromised by genome instability may fail to maintain homeostasis, and (iii) the allocation of resources to maintenance processes, as influenced by signalling pathways (e.g. IGF, TOR), may both cause and be caused by changes in internal and external state. A further set of interesting questions about evolution of patterns of ageing and longevity arises from the fact that some organisms are exposed to unpredictable and/or varying environments. It is therefore at least plausible that in some species there has evolved a capacity for adaptive plasticity whereby the allocation of metabolic resources between maintenance, growth and reproduction may be altered, e.g. in times of stress or famine. Examples having direct consequences for ageing and longevity are seen in cases of increased lifespan following dietary restriction or where adverse environments evoke the formation of long-lived, stress resistant dispersal forms such as the *dauer* larva in *Caenorhabditis elegans*. Similar principles can also inform our understanding of the differences in ageing and longevity that are seen between workers and queens in social species such as bees and ants.

## **A century of climate and land-use impacts on the metacommunity dynamics of California birds and mammals**

BEISSINGER STEVEN R

Department of Environmental Science, Policy & Management, and Museum of Vertebrate Zoology, University of California, 130 Mulford Hall, Berkeley, CA 94720-3110, USA; beis@berkeley.edu

Forecasts of global climate change on biodiversity typically relate recent species' occurrences with climate variables to project future ranges, often with alarming conclusions. While evidence supports temperature-driven range expansions poleward, there is substantial variation in responses among species. Assessments have been limited by evaluating range change over short time spans, or by not accounting for the confounding effects of land-use change and false absences. Historical resurveys – where biodiversity surveys from the past are resampled – provide important opportunities to understand the influence of 20<sup>th</sup> century environmental change on biodiversity and to establish new benchmarks for understanding future change. Here I report on a 15-year effort to resample locations throughout California that Joseph Grinnell and colleagues originally surveyed for birds and mammals from 1908-1939. This includes resurveys of small mammals and birds across 4500-m elevational (spanning the highest and lowest points in the continental US) and 1250-km latitudinal gradients that comprise California on landscapes of long-protected, iconic national parks (e.g., Yosemite and Death Valley) as well as other public and private lands. Highly variable climates and levels of climate change over the past century provide an important opportunity to decompose the effects of local changes in temperature, precipitation and land use on site-level turnover of species. Using occupancy modelling to control for variation in detectability, we found substantial heterogeneity in elevational range shifts among bird and mammal species and among regions. Local colonisations and extinctions of birds followed trends predicted by their climatic niche, but some species tracked changes in temperature while others tracked precipitation. Birds that responded strongly to climate change also responded strongly to land use change. Metacommunity dynamics suggest a strong potential for a slow erosion of species richness.

## **Life in the arid lands: ecological challenges and behavioural flexibility in wild baboons**

ALBERTS SUSAN

Duke University, Departments of Evolutionary Anthropology and Biology, Box 90338, Durham, NC, 27708, USA; alberts@duke.edu

Baboons are highly successful generalist foragers that inhabit a wide range of habitats throughout Africa, from near-desert to moist forest. This ecological flexibility stems from their behavioural plasticity, and at the same time exposes them to often-challenging environments. In this presentation, I focus on a single well-studied population of baboons living in a semi-arid and highly unpredictable environment, the baboons of the Amboseli basin in southern Kenya. Using four decades of data on life history and behaviour, I describe multiple ways in which environmental conditions – including early life and immediate environments – can impose constraints on reproduction and survival. I also examine the opportunities that behavioural and life history flexibility can bring in the face of challenging environments. Specifically, I examine how behavioural traits influence reproduction and survival, sometimes in ways that counter environmental effects and sometimes in ways that exaggerate such effects. In other words, the very behavioural flexibility and complexity that baboons use to confront and break free of environmental constraints imposes its own costs and limitations.

## **Application of genomic approaches to the management of *ex situ* populations of endangered species**

KOEPFLI KLAUS-PETER

Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, 3001 Connecticut Avenue NW, Washington, D.C., 20008 USA;  
Dobzhansky Center for Genome Bioinformatics, Saint Petersburg State University, 41A Sredniy Prospekt, Saint Petersburg 199034, RUSSIA; klauspeter.koepfli527@gmail.com

A significant challenge to adequately managing animals in *ex situ* populations such as those found in zoo settings is the ability to retain adequate genetic diversity to ensure species integrity, evolutionary potential, health and reproduction. Especially important is identifying males and females that are unrelated to avoid the consequences of inbreeding. Historically, managers have relied on monitoring lineages through a studbook, a process that can be stymied by gaps in data or assumptions and best guesses of animal inter-relatedness. The advent of whole genome analyses through next generation sequencing technologies has the potential to improve our ability to manage both *ex situ* and *in situ* populations of wildlife. Genome-wide assessment of genetic diversity permits a detailed examination of admixture, demography, inbreeding, and kinship among individuals, thereby facilitating the genetic monitoring of populations over time.

In this talk, I will provide an overview of our program to develop and apply data derived from whole genome sequences to the management of *ex situ* populations of several endangered species. Species-specific genotyping arrays based on thousands of single nucleotide polymorphisms (SNPs) can be applied to determine the amount and distribution of genome-wide diversity and estimate relatedness of individuals and populations, thereby facilitating a more holistic management of meta-populations derived from different *ex situ* settings. Furthermore, genes and pathways associated with reproduction can be potentially identified and analysed to better understand differential reproductive fitness among individuals. Decreasing financial costs make it likely that genomic data will increasingly become an indispensable resource for conservation management of many species facing population declines.

## **PARALLEL SESSION AND WORKSHOP SUMMARIES**

### **Parallel session I: Conservation**

SANTOS NUNO<sup>1</sup>, EAST MARION L<sup>2</sup>

<sup>1</sup>CIBIO – Research Center in Biodiversity and Genetic Resources, University of Porto, Campus de Vairao, 4485-661 Vairao, PORTUGAL; pygargusv@sapo.pt

<sup>2</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; east@izw-berlin.de

Human activities have caused an alarming loss of biodiversity, large reductions in wildlife populations, rising human-wildlife conflict, and the degradation of both habitats and ecological processes. This workshop aims to discuss these conservation problems and the approaches used to alleviate them.

### **Parallel session II: Urban wildlife ecology**

RILEY SETH PD<sup>1</sup>, KRAMER-SCHADT STEPHANIE<sup>2</sup>

<sup>1</sup>National Park Service, Santa Monica Mountains National Recreation Area, 401 W. Hillcrest Dr. Thousand Oaks, CA 91360, USA; seth\_riley@nps.gov

<sup>2</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; kramer@izw-berlin.de

Urban areas are rapidly increasing worldwide and lead to the transformation of habitat for wildlife. On the one hand, novel ecosystems may arise, e.g. by offering new food resources, while on the other hand the human-wildlife interface increases through habitat fragmentation and expansion of developed areas. These processes not only change the contact zones between humans and wildlife leading to human-wildlife conflicts, but also alter transmission pathways for zoonotic pathogens or emerging infectious diseases. It is therefore of paramount importance to understand the adaptive strategies of wildlife species to urban environments to contribute to a reasonable wildlife management. This workshop aims to provide an overview of current work done in the relatively young field of urban wildlife ecology by focusing on behavioural and physiological adaptations (movement behaviour and habitat selection, genetic structure, resource use) of wildlife to urban environments, stressors and selective pressures to wildlife in cities as well as on infectious diseases of wildlife at the human-wildlife interface.

### **Parallel session III: Environmental epigenetics**

WEYRICH ALEXANDRA<sup>1</sup>, SKINNER MICHAEL K<sup>2</sup>, SOEN YOAV<sup>3</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; weyrich@izw-berlin.de

<sup>2</sup>Center for Reproductive Biology, School of Biological Sciences, Washington State University, Abelson 507, Pullman, WA 99164-4236, USA; skinner@wsu.edu

<sup>3</sup>Weizmann Institute of Science, 234 Herzl Street, Rehovot, 7610001, ISRAEL; yoavs@weizmann.ac.il

Epigenetic modifications regulate gene expression and convey environmental information to subsequent generations via parental germ lines. This workshop will focus on the mammalian epigenetic response to environmental changes such as toxins, stress, nutrition, and temperature and its transmission to subsequent generations. Epigenetic transgenerational inheritance of such experiences can be beneficial in preparing the offspring to novel environments, but can also multiply the risk of diseases. These novel findings (and others) are suggesting the importance of non-genetic factors for rapid and lasting adaptation processes, and their importance in epidemiology, wildlife conservation and evolution.

### **Parallel session IV: Ecophysiology of wildlife**

BLUMSTEIN DANIEL T<sup>1</sup>, ORTMANN SYLVIA<sup>2</sup>

<sup>1</sup>Department of Ecology and Evolutionary Biology, 621 Young Drive South, University of California, Los Angeles, CA 90095-1606, USA; marmots@ucla.edu

<sup>2</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Dept. of Evolutionary Ecology, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; ortmann@izw-berlin.de

Ecophysiology studies the adaptation an organism's physiology to environmental conditions and the reaction to changes in environmental conditions. Availability, assimilation and allocation of food and/or energy is of vital importance for several aspects of the biology and life-history of animals, e.g. colonization of extreme habitats and niche separation, population dynamics and social systems, activity and habitat use, growth and reproduction. This workshop reviews various aspects of ecophysiology such as adaptation to seasonality, management of energy resources and energy reserves, acquisition and processing of food, and the effect of climate change on physiological adaptations in wildlife. Recent findings will be presented of research from field and laboratory studies and new techniques will be introduced, which hopefully stimulates discussion and encourages young scientists to work in this field.

## **Workshop: Cryopreservation of gametes and embryos for conservation breeding**

MÜLLER KARIN<sup>1</sup>, SCHENKEL JOHANNES<sup>2</sup>, JEWGENOW KATARINA<sup>1</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; mueller@izw-berlin.de

<sup>2</sup>German Cancer Research Center, Cryopreservation W430, Im Neuenheimer Feld 280, 69120 Heidelberg, GERMANY; j.schenkel@dkfz-heidelberg.de

Cryobanking of gametes and reproductive tissues becomes increasingly important to assist reproduction in endangered species and secure valuable genetic sources for future actions. Based on the extensive routine experiences in cryobanking mouse gametes and embryos for medical research, we address important aspects concerning preservation procedures, storage conditions and facilities. Future cryobanking options within the zoo and wildlife community will be discussed.

Considering the fact that most samples are recruited under field conditions, the application of simple cryopreservation methods with low operating expense are in focus. Cell-, tissue-, individual- and species-specific peculiarities still constitute challenges for cryopreservation of reproductive cells and tissues and require intense research. Here we particularly consider protection and repair mechanisms of membrane lipids.

## **Workshop: Wildlife detection dogs**

BERGER ANNE<sup>1,2</sup>, GRIMM-SEYFARTH ANNEGRET<sup>2,3</sup>, MAUSBACH JELENA<sup>2,4,5</sup>, CLYNE ARAN<sup>6</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; berger@izw-berlin.de

<sup>2</sup>Wildlife Detection Dogs e.V., www.wildlifedetectiondogs.org, Sofienstraße 5, 74867 Neunkirchen, GERMANY

<sup>3</sup>UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstr. 15, 04318 Leipzig, GERMANY; annegret.grimm@ufz.de

<sup>4</sup>ETH Zürich, Eawag, Überland Straße 133, 8600 Dübendorf, SWITZERLAND; jelena.mausbach@gmx.de

<sup>5</sup>Stiftung Fledermausschutz Schweiz, Zürichbergstraße 221, 8044 Zürich, SWITZERLAND

<sup>6</sup>Wagtail UK Ltd and Conservation Dogs, PO Box 143, Flint, CH6 9DF, UK

The use of wildlife detection dogs currently experiences an increasing popularity in research, monitoring and nature conservation especially by improving the detection of cryptic species. However, the use of wildlife detection dogs requires guidelines and certifications in order to ensure high quality standards and documented results and, consequently, to get broad official approval.

After giving a general introduction and overview about how wildlife detection dogs can assist researchers in data collection and their use worldwide, we will discuss potential requirements and quality standards with European experts working in that area.

## **PARALLEL SESSION AND WORKSHOP INTRODUCTORY TALKS**

### **Parallel session I: Conservation**

#### **Wolf conservation in Portugal: contributions from zoological medicine and physiology**

SANTOS NUNO

CIBIO – Research Center in Biodiversity and Genetic Resources, University of Porto, Campus de Vairao, 4485-661 Vairao, PORTUGAL; pygargusv@sapo.pt

Wolf populations in Portugal experienced a severe decline during the 20<sup>th</sup> century, having disappeared from 80 % of the former distribution range. After the species was fully protected in the 1980's and compensation for depredation on livestock was introduced, the distribution stabilized, is locally increasing and local densities can be high. The main conservation concern, like elsewhere in Europe, is management of the conflict with humans. The situation of a demographically isolated sub-population south of Douro river is particularly worrisome.

The depredation compensation scheme pays several thousand euros annually to livestock owners, including incentives for improved protection of the herds. As part of an experimental effort to reduce conflict with livestock owners, veterinary treatment of animals injured during predation events was provided free of charge in a region of north western Portugal, where the wolf diet is > 90 % livestock.

In the last two decades all wolves found dead in Portugal have been processed through a centralized scheme, aiming to determine the causes of death and provide biological samples to research institutions. As of today, 98 wolves were necropsied and the main causes of death were vehicle run-over (31 %), snares (18 %), shooting (18 %), poisoning (4 %), infectious disease (3 %) and interaction with canids (3 %). All known mortality events of radio-tagged wolves (n = 10) were of anthropogenic origin, further highlighting the high poaching rates this protected large carnivore endures in human-dominated landscapes. The biological samples collected from these wolf carcasses allowed to uncover localized hybridization events with dogs, apparent congenital bone defects in 2 individuals from the south Douro and a lower genetic variability in this sub-population. Exposure to several infectious agents was also determined, particularly canine parvovirus and canine distemper virus, with an apparent increased risk in the south Douro sub-population.

In the last decade 23 wolves were captured and GPS/GSM collared for ecological research, allowing to characterise the acute capture stress response in this species. This research showed that reducing the duration of restraint on leg-hold snares by using remote satellite trap-alarms coupled to the traps dampens the alterations in several physiological and behavioural stress mediators, and should become standard practice in the capture of free-ranging wolves.

## **Parallel session II: Urban wildlife ecology**

### **Effects of urbanization and fragmentation on wildlife populations in a National Park in Southern California, from newts to mountain lions**

RILEY SETH PD

National Park Service, Santa Monica Mountains National Recreation Area, 401 W. Hillcrest Dr. Thousand Oaks, CA 91360, USA; seth\_riley@nps.gov

The role of national parks is to preserve the natural resources present in the parks, including the wildlife resources. At Santa Monica Mountains National Recreation Area (SMMNRA), a national park next to Los Angeles, the primary challenges for effective wildlife conservation are understanding and mitigating the effects of urbanization, including habitat loss and fragmentation. We have been studying a number of different wildlife communities in and around the park for 15-20 years. Southern California is home to many diverse wildlife communities, and much of this diversity still exists in the park, despite intense and widespread development in surrounding areas. However, we have also documented multiple threats to wildlife communities in this urban landscape. Stream amphibian populations are threatened by altered stream structure and permanence, which allows exotic predators to persist in urbanized watersheds. Fragmentation results in the loss of terrestrial reptile species from smaller habitat patches, particularly larger snakes. For some of the more widespread species that remain even in smaller patches (e.g., western fence lizards), we have seen that fragmentation is already associated with significant genetic differentiation. These genetic effects of fragmentation are evident even in a bird, an animal that we might presume could fly over urban barriers to mitigate the effects of development. Finally, for the most wide-ranging, and therefore potentially vulnerable group, mammalian carnivores, we have seen that species such as coyotes, bobcats, and mountain lions are surprisingly adaptable to development. Yet carnivores too face significant threats. Toxicants, specifically anticoagulant rodenticides, are present at high levels in all three species, and these toxicants can have significant population-level effects. Finally, roads and development form major barriers to movement for carnivores, leading to significant reductions in gene flow. Our long-term studies have shown that there is great value in preserving urban wildlife communities in Southern California, but that significant challenges remain.

### **Parallel session III: Environmental epigenetics**

#### **Impacts of environmentally induced epigenetic transgenerational inheritance on disease, phenotype variation and evolution: ancestral ghosts in your genome**

SKINNER MICHAEL K

Center for Reproductive Biology, School of Biological Sciences, Washington State University, Abelson 507, Pullman, WA 99164-4236, USA; skinner@wsu.edu

Transgenerational effects of environmental toxicants, nutrition or stress significantly amplify the biological impacts and health hazards of these exposures. One of the most sensitive periods to exposure is during fetal gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation occurs. Previous studies have shown that toxicants can cause an increase in adult onset disease such as infertility, prostate, ovary and kidney disease, cancers and obesity. Interestingly, this effect is transgenerational (F1, F2, F3 and F4 generations) and hypothesized to be due to a permanent (imprinted) altered epimutation of the germ line. The transgenerational epigenetic mechanism appears to involve the actions of an environmental compound at the time of sex determination to permanently alter the epigenetic (e.g. DNA methylation) programming of the germ line that then alters the transcriptomes of developing organs to induce disease susceptibility and development transgenerationally. In addition to DNA methylation, alterations in sperm ncRNAs and histone retention have also been observed. A variety of different environmental compounds have been shown to induce this epigenetic transgenerational inheritance of disease including: fungicide vinclozolin, plastics BPA and phthalates, pesticides, DDT, dioxin and hydrocarbons. Interestingly, exposure specific epigenetic alterations were observed between the specific toxicants. Similar observations have been observed in other species such as alligators, Darwin's finches and New Zealand mud snails. The suggestion that environmental factors can reprogram the germ line to induce epigenetic transgenerational inheritance of disease and phenotypic variation is a new paradigm in disease aetiology that is also relevant to other areas of biology such as wildlife conservation and evolution.

## **Paternal epigenetic effects in response to environmental change in wild guinea pigs**

WEYRICH ALEXANDRA<sup>1</sup>, LENZ DORINA<sup>1</sup>, JESCHEK MARIE<sup>1,2</sup>, SCHRAPERS KATHARINA T<sup>1,3</sup>, HUNG CHUNG TZU<sup>4</sup>, RÜBENSAM KATHRIN<sup>1</sup>, JEWGENOW KATARINA<sup>1</sup>, FICKEL JÖRNS<sup>1,5</sup>

<sup>1</sup>Leibniz-Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; weyrich@izw-berlin.de

<sup>2</sup>Berlin Center for Genomics in Biodiversity Research; Königin-Luise-Str. 6-8; 14195 Berlin, GERMANY

<sup>3</sup>PerformaNat GmbH; Hohentwielsteig 6, 14163 Berlin; GERMANY

<sup>4</sup>Zymoresearch, EpiQuest, 17062 Murphy Ave., Irvine, CA 92614, USA

<sup>5</sup>Institute for Biochemistry and Biology, Potsdam University, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, GERMANY

Temperatures are globally rising, causing a shift in vegetation. Epigenetic modifications, such as DNA methylation is a mechanism conveying environmental information through generations. To address the paternal impact on the offspring's adaptability to environmental changes we studied the epigenetic response 1) to temperature increase in F0 and F1 male wild guinea pigs, and 2) in a second experiment to changes in nutrition composition in F1 males. In most wild mammal species, males are the dispersing sex and have to cope more rapidly to differing habitats than the philopatric females. We exposed five male wild guinea pigs for two months 10 °C increase in temperature (to 30 °C) and fed a second group of five male wild guinea pigs for two months with a protein diet. We then analysed DNA methylation changes in livers and testes. We detected differentially methylated regions (DMRs) in annotated regions ('annotated DMRs') in sons sired before and after their fathers' treatments. Network analysis of both experiments identified mostly treatment-dependent genes with major metabolic functions. Our results indicated a 'heritable epigenetic response' to the diet change of the fathers, which was transmitted paternally to the F1 and potentially even to the F2 generation.

## **Adaptation by Natural Improvisation: A theory of individual-specific adaptation**

SOEN YOAV

Weizmann Institute of Science, 234 Herzl Street, Rehovot, 7610001, ISRAEL;  
yoavs@weizmann.ac.il

Every individual organism is endlessly challenged by internal perturbations in its somatic genome, epigenome and gut microbiome. This gives rise to an unimaginable number of novel combinations of changes that are unique to each individual. Overcoming such a high frequency of individual-specific perturbations cannot be explained solely by natural selection (which operates only on a population level). How individual-specific adaptation comes about is, thus, a highly fundamental open question.

To address this question we have previously proposed a theory of individual-specific adaptation, based on a new principle of organization, complementary to natural selection. This concept (termed “Adaptive Improvisation”) explains how biased random variations that emerge during the lifetime of every individual can safely and rapidly confer a wide range of newly-forming adaptations. This applies to any kind of variation (epigenetic, physiologic, symbiotic, genetic, etc.) and explains how these variations can self-organize into new adaptive states. In some of the specific realizations of this concept, the newly acquired adaptations are also heritable, thus enabling further improvement and assimilation in a few generations. Experimental and theoretical evaluation of this theory provide evidence supporting a capacity to adapt by exploratory dynamics over time in a single generation. I will describe this Lamarckian theory and present new supportive evidence (unpublished).

## **Parallel session IV: Ecophysiology of wildlife**

### **Social buffering of predator-induced stressors in marmots**

BLUMSTEIN DANIEL T

Department of Ecology and Evolutionary Biology, 621 Young Drive South, University of California, Los Angeles, CA 90095-1606, USA; marmots@ucla.edu

Individuals embedded in strong social networks may live longer, healthier lives. Social buffering and support is widely reported in humans and in some primates and rodents. I will discuss lessons from our long-term study of yellow-bellied marmots that documents the importance of predators to marmots. Predator pressure varies spatially and temporally. I discuss the consequences of physiological stress on marmots' propensity to emit alarm calls and the acoustic structure of these alarm calls. Marmots from high predator pressure sites have enhanced glucocorticoid levels and predator presence is associated with a suite of life history responses. Meaningful social relationships may buffer females from these stressors, which may be important because pups from stressed mothers have reduced survival.

## **Workshop: Cryopreservation of gametes and embryos for conservation breeding**

### **Cryopreservation of mutant mice**

SCHENKEL JOHANNES

German Cancer Research Center Heidelberg, Cryopreservation W430, Im Neuenheimer Feld 280, 69120 Heidelberg, GERMANY; j.schenkel@dkfz-heidelberg.de

Due to the availability of many genetically modified (GM) lines, mice are the most important laboratory animals used in bio-medical basic research. Generating and characterizing GM lines involves tremendous effort, their number is increasing rapidly. Several limitations have to be considered if working with these animals: small colonies, the continued danger of loss, a limited breeding-success, interchanges between facilities, the need to keep those lines in stock, and their major (scientific) value. The cryopreservation of pre-implantation embryos or spermatozoa is a common approach to keep GM mouse lines available at any time while avoiding or at least dramatically reducing the need of living animals. The breeding of a line can be discontinued following a sufficient cryopreservation. Strict quality-assessments and safe long-term storage conditions are mandatory to keep the capacity to recover a line, also after years.

Cryopreservation of pre-implantation embryos consumes many embryo-donors but leads to an easy and secure recovery. Therefore, all procedures must be optimized, negative factors determined and eliminated. Here, assessment strategies and parameters influencing embryo yields were investigated, e.g. the genetic background, the donor-age, the housing conditions; pheromones, mating frequency, hygienic conditions; the environmental factors (annual rhythms, humidity, noise, temperature), or diet. To guarantee an efficient recovery the genetic stability, the revitalization rate, the sex ratio, and the apoptotic behaviour of frozen/thawed preimplantation embryos depending on their developmental stage and the storage-period (up to 15 years) were elucidated as well as the hygienic state of the cryopreserved samples.

Sampling and cryopreservation of spermatozoa is relatively simple. However, an in vitro-fertilisation (IVF) is required following the revitalization. This animal consuming technique exhibits varying success and is not available for all genetic backgrounds. To examine the quality a frozen sample will be subjected to IVF. Alternatively, the mobility and motility of the thawed spermatozoa will be examined by using fluorescence microscopy or by scoring.

To keep the information about the cryopreserved samples upright, a powerful data management including data bases and meeting the international nomenclature rules is mandatory.

Cryopreservation allows a safe storage of mutant mouse samples. These procedures applied can affect the progeny. Cryopreservation and advanced sampling and assessment-strategies are contributing to the reduction of animals and subsequently to the “3Rs”.

## **Workshop: Wildlife detection dogs**

### **Chances and challenges of using wildlife detection dogs in nature conservation and wildlife research projects**

BERGER ANNE<sup>1,5</sup>, GRIMM-SEYFARTH ANNEGRET<sup>2,5</sup>, MAUSBACH JELENA<sup>3,4,5</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Dept. of Evolutionary Ecology, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; berger@izw-berlin.de

<sup>2</sup>UFZ-Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstraße 15, 04318 Leipzig, GERMANY

<sup>3</sup>ETH Zürich, Eawag, Überland Straße 133, 8600 Dübendorf, SWITZERLAND

<sup>4</sup>Stiftung Fledermausschutz Schweiz, Zürichbergstraße 221, 8044 Zürich, SWITZERLAND

<sup>5</sup>Wildlife Detection Dogs e.V., www.wildlifedetectiondogs.org, Sofienstraße 5, 74867 Neunkirchen, GERMANY

The highly developed olfaction in combination with a very good learning ability and the willingness to cooperate with humans qualifies dogs for a wide range of tasks. Thus, the use of so called “wildlife detection dogs” in nature conservation and wildlife research is already an effective and successfully applied method in many regions of the world. They are used for: 1) the detection of rare, protected, elusive or conflict-laden species, 2) the detection of faeces (“scat detection dogs”) which then can be further analysed for nutrition, hormones, endoparasites or genetics, 3) the detection of samples (faeces, hairs, feathers etc.) of certain species in order to avoid confusion with similar or strongly related other species, 4) the precise detection of harmful or invasive species, 5) the detection of wildlife smuggling and wildlife contrabands, 6) surveys on damage caused by wind power stations, 7) wildlife research projects (where dogs could also be used to detect the sex or reproductive state of the detected individual).

In most studies, the success rate when using trained dogs is significantly higher than when working without a dog; professionally trained dogs were proven to be more effective and at the end more cost-effective than other conventional methods. On the other hand, previous experiences showed that the detection rate is influenced by factors like the weather, the terrain topography and the competence of the dog handler. Frequent challenges using wildlife detection dogs are for instance: 1) to get and store odour samples of rare, protected or invasive species for dog training, 2) the shortness of projects/contracts versus the time to train the dog, 3) long-term monitoring projects versus the life span of a dog, 4) availability of trained dogs at soonest or at certain times or seasons, 5) the diversity of project tasks versus the specificity of dog training, 6) lack of official approvals of wildlife detection dogs, 7) lacking valid certificates of dog performance, 8) comparability of the dogs’ performances.

In general, the use of detection dogs in nature conservation is clearly more popular in America, Africa, Australia and New Zealand than in Europe. In order to use wildlife detection dogs scientifically and systematically, as well as to achieve societal recognition, national and international standards and certification options for the proof of their performance have to be developed and implemented.

## CONTRIBUTED PAPERS

**Selection of an avian reference genome for mapping and assembly of the Cape Vulture next generation sequencing data**

ADAWAREN EMMANUEL<sup>1</sup>, DU PLESSIS MORNE<sup>2</sup>, MUKANDIWA LILIAN<sup>1</sup>, SULEMAN ESSA<sup>2</sup>, NAIDOO VINNY<sup>1</sup>

<sup>1</sup>Faculty of Veterinary Science, Department of Paraclinical Science, University of Pretoria, M35 Onderstepoort Soutpan Road Pretoria, 0110, SOUTH AFRICA; adawarenvet1@yahoo.com

<sup>2</sup>National Zoological Gardens of South Africa, Research and Scientific Services Department, P.O. Box 754, 232 Boom Street Pretoria, 0001, SOUTH AFRICA

The Cape Vulture (*Gyps coprotheres*) is a large sized scavenger belonging to the Accipitridae family of the Old World vultures and plays an important role of “cleaning” the ecosystem. Old World vultures are facing extinction due to multiple threats such as collision with wind turbines and electric cables, malicious poisoning, trade in specific appendages for traditional medicine and most recently from the ingestion of diclofenac contaminated carcasses in the Asian subcontinent (OAKS *et al.*, 2004). Pathognomonic signs of toxicity and pharmacokinetic parameters have been established in Cape Vulture and other related *Gyps* species (SWAN *et al.*, 2006). Toxicity is due to saturation of the metabolic enzymes and/or possible pharmacogenetic defects in one or more Cytochrome P450 (CYP) genes, possibly in CYP2C9, known for hydroxylation of lipophilic diclofenac into water-soluble 4-hydroxydiclofenac in humans (NAIDOO *et al.*, 2010). Currently, there is no sequence information available for this family of genes in the Cape Vulture making it difficult to conduct pharmacogenetic studies for diclofenac toxicity. Therefore, Next Generation Sequencing (NGS) of the Cape Vulture was performed on the Ion Torrent S5 platform. The Cape Vulture draft assembly was generated by combining data from de novo assembly with data generated from mapping of the reads to the Turkey vulture (*Cathartes aura*) as the reference. Several putative CYP gene sequences were obtained from the draft assembly of the Cape Vulture NGS data. NCBI BLAST of these sequences performed against other avian sequences in GenBank showed similarity of generally less than 70 %. It was therefore hypothesised that due to the evolutionary divergence between the New and Old World vultures (JOHNSON *et al.*, 2016; SEIBOLD and HELBIG, 1995) the Turkey vulture was an unsuitable reference for mapping. A search for a more appropriate avian reference genome was necessary. To achieve this, phylogenetic analysis was undertaken in MEGA v7.0 for three mitochondrial genes (Cytochrome b (COB), Cytochrome c oxidase 1 (COX1) and NADH dehydrogenase 2 (NAD2)) and one nuclear encoded gene (beta-fibrinogen 7 (BFI7)) from GenBank for the Cape Vulture and other birds for which complete genome were available. The results showed that the Golden Eagle (*Aquila chrysaetos*) was more closely related to the Cape Vulture than the Turkey Vulture, suggesting that it may be a more appropriate reference for mapping and assembly of the Cape vulture NGS data.

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## **Homologous and heterologous feline embryos produced *in vitro* with frozen-thawed semen of domestic cat (*Felis silvestris catus*) and Far-Eastern wildcat (*Prionailurus bengalensis euptilurus*)**

AMSTISLAVSKY SERGEI<sup>1</sup>, BRUSENTSEV EUGENY<sup>1</sup>, MOKROUSOVA VALENTINA<sup>1</sup>, KIZILOVA ELENA<sup>1</sup>, KOZHEVNIKOVA VALERIA<sup>1</sup>, ROZHKOVA IRINA<sup>1</sup>, NAIDENKO SERGEI<sup>2</sup>

<sup>1</sup>Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Division, prosp. Lavrentjeva 10, 630090 Novosibirsk, RUSSIA; amstis@yandex.ru

<sup>2</sup>A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskij prosp. 33, 119334 Moscow, RUSSIA

Far-Eastern wildcat (*Prionailurus bengalensis euptilurus*) is a felid inhabiting the Far East of Russia. The objective of this study was to explore the possibility of producing *in vitro*-derived embryos by the *in vitro* fertilisation of *in vitro* matured domestic cat oocytes with frozen-thawed semen of domestic cat and Far-Eastern wildcat. Sperm collection was performed by electroejaculation in the latter. Domestic cat spermatozoa were obtained from epididymis collected from the local vet clinics in Novosibirsk. Semen was diluted with CaniPlus Freeze (CPF) (Minitube, Germany), equilibrated at +4 °C and frozen in 0.25 ml straws (Cryo Bio System, France) in a styrofoam container, where straws were placed horizontally on a metal rack 5 cm above the liquid nitrogen surface for 20 min, and plunged into liquid nitrogen thereafter. For thawing straws were held 10 sec at RT, followed by 30 sec in a water bath of +37.0 °C. The viability of spermatozoa was evaluated either by double staining with SYBR Green I/PI and the subsequent fluorescence microscopy or by VitalScreen test combined with light microscopy. Sperm morphology was assessed by light microscopy from the samples of frozen-thawed spermatozoa in each of the male studied. *In vitro* matured domestic cat oocytes were *in vitro* fertilised in 50 µl drops in Ham's F-10 supplemented with 5 % of FCS with  $1 \times 10^6$  spermatozoa per ml. Semen survival after cryopreservation was  $38.2 \pm 3.0$  % for domestic cat and  $36.7 \pm 6.5$  % for Far-Eastern wildcat. The viability of semen before freezing was  $56.5 \pm 9.7$  % and  $60.0 \pm 9.4$  % correspondingly. Difference between pre-freeze and post-freeze viability of semen was insignificant for both species. The most frequent anomaly for spermatozoa of domestic cat and Far-Eastern wildcat were flagella defects. The rate of embryo development up to morula stage was  $30.0 \pm 5.5$  % after homologous and  $35.5 \pm 15.0$  % after heterologous IVF. Conclusion: Epididymal and ejaculatory felid semen was successfully frozen with CPF. Our data report the first successful attempt of *in-vitro*-derived embryos produced after heterologous IVF of domestic cat oocytes with frozen-thawed semen of Far-Eastern wildcat.

This study was supported by the Russian Foundation for Basic Research (Project No. 15-04-03258).

## **Seal rehabilitation: motherless pups, lungworms and other seal diseases**

BAHR JANINE, VAN GEMMERT ANDRÉ

Robbenzentrum Föhr, Achtern Diek 5, 25938 Wyk auf Föhr, GERMANY;  
janine\_insel@hotmail.com

There are different marine mammal rehabilitation centres around the world, which receive harbour seals (*Phoca Vitulina*): mostly motherless pups, animals with different types of trauma, those entangled in nets or other human-caused injuries. In the last three years there has been an increasing number of lungworm infections in seals in the European North Sea, especially in the Wadden Sea area, including harbour and increasingly also in grey seals. There is a minimum of two species of worms including *Ostrongylus circumlitus* and *Parafilaroides gymnurus*. The reasons for infection are very different, but every time it could be highly dangerous. Lungworms are often found in wildlife animals. But the high number of infections and the kind of such heavy infections are not seen before. So it seems a really bad infection that could decrease the whole population. The presentation will also show the possibility of treatment of the infected animals. At the moment, the reasons for the increase of this kind of infections are not totally researched. Overfishing, warming of the atmosphere or maybe the growing of environmental pollution, the experts are sure that the reason is caused by humans and their way of living. Studies from Great Britain show that the immune system of even good weight seals is very sensible and delicate. Also, the whole procedure of a complete rehab will be explained, from the first step and first aid to the release.

## **Nesting behavior of the European hedgehog (*Erinaceus europaeus*, Linnaeus, 1758) under direct anthropogenic influences**

BARTHEL LEON MF<sup>1</sup>, OLTMANN FEONA CI<sup>2</sup>, BERGER ANNE<sup>1</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Dept. of Evolutionary Ecology, Alfred-Kowalke-Straße 17, 10315, Berlin, GERMANY; barthel@izw-berlin.de

<sup>2</sup>Van Hall Larenstein University of Applied Sciences (VHL), Agora 1, 8934 CJ, Leeuwarden, THE NETHERLANDS

Urbanization is a global process of Anthropocene that also affects wildlife. In an urban environment, animals have to cope with increased direct and indirect human influences on their habitat. Beside indirect and mostly slow changes like light pollution or climate change, there are also a lot of direct and mostly fast changes which occur within days or weeks and, thus, affect the animals daily life. The reaction to those changes of habitat is important for survival of the individual or species. Related to this framework, we investigated the effect of a festival on the nesting behavior of European hedgehogs in an urban park. As a nocturnal insectivore species, hedgehogs rely on nest during day to sleep and rest.

Festival took place on two days (added 18 days of construction and deconstruction work) having about 140,000 festival visitors. During the research period from August until September 2016, 17 hedgehogs (9 male, 8 female) were captured within the festival area and fitted with a VHF radio-transmitter. Daily controls of the nest use of every tracked hedgehog were carried out. Data was analysed with respect on the effect of the festival on the nesting behaviour comparing periods before and during the festival respectively construction work.

Survival analyses of the nest use show a different result for male and female. During the festival, male hedgehogs significantly decreased their time using the same nest, while females did not change the nests more than before the festival. Taking into account that some of the tracked female hedgehogs did care for hoglets during that time could be an explanation why they were not able to change the nest.

In summary, male hedgehogs changed their nests more frequent during the whole festival (including periods of construction and deconstruction), while females showed no significant difference.

It is not clear how the hedgehog population is or is not affected in the longterm or energy metabolism by those big events but further analyses in the project “hedgehogs in Berlin” will investigate also the influence on the movement and the rhythmic behaviour using data from GPS and accelerometer devices to get more information about the short term reactions.

## Tolerance of domestic cat epididymal sperm for different equilibration conditions prior to cryopreservation

BASHAWAT MOHAMMAD, FRANZ CHRISTIANE, MÜLLER KARIN

Leibniz Institute for Zoo and Wildlife Research, Dept. of Reproduction Biology, Alfred-Kowalke-Str. 17, D-10315 Berlin, GERMANY; bashawat@izw-berlin.de

The term equilibration is used to describe the osmotic adaptation of sperm to the presence of cryoprotectants as well as the adaptation of sperm membranes to lower super-zero temperatures immediately before freezing. A slow and long (2 h) equilibration to +4 °C is often applied to diluted semen in a variety of species. Recently, we could show that a slow but shorter (40 min) equilibration may yield satisfying results in the epididymal semen of domestic cat (KLAUS *et al.*, 2016) and in ejaculates of African lion (LUTHER *et al.*, 2017). To further facilitate equilibration under field conditions, we tested the following schemes.

Epididymal sperm of domestic cat (n = 4) were diluted at ~22 °C (room temperature), in TestG (TES-Tris-Fructose based extender), subsequently four different equilibration treatments were administrated: 1) protected slow cooling performed in a refrigerator (+6 °C) for 40 min in water jacket design (control), 2) unprotected fast cooling performed in a refrigerator for 15 min (fridge), 3) equilibration at ~22 °C (RT) or 4) at +38 °C in a block heater (+38 °C) each for 15 min, Motion parameters were determined by AndroVision™ (Minitüb, Germany) ten minutes after thawing as well as after subsequent washing and incubation at +38 °C for ten minutes, one, two, and three hours. No difference was revealed between the treatments (p > 0.05). For control, fridge, RT, and +38 °C treatments, the percentages of total and progressive motility were (49.62 ± 5.44, 54.92 ± 5.44, 45.52 ± 5.44, 47.86 ± 5.44 %) and (36.56 ± 5.02, 40.86 ± 5.02, 35.80 ± 5.02, 33.970 ± 5.02 % ) after washing and ten minutes incubation, the corresponding percentages were (23.14 ± 5.44, 22.67 ± 6.32, 25.28 ± 5.44, 22.67 ± 5.44 %) and (10.25 ± 5.029, 10.08 ± 5.84, 15.56 ± 5.029, 10.85 ± 5.02 %) after three hours of incubation respectively.

These observations indicate that epididymal domestic cat sperm even tolerate high or moderate temperatures (+38 °C, +22 °C) for equilibration. Whether this may be extended to other felid species remains to be proven, but it would help to simplify the protocol and curtail the time required for cryopreservation when valuable and often very rare gametes are only available in field. Moreover, further experiments are necessary to assess the fertilising competence of these sperm.

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## **Long-term patterning of reproduction in the global historical captive population of the Lion-tailed macaque (*Macaca silenus*)**

BEGUM NILOFER<sup>1,3</sup>, KAUMANN WERNER<sup>2</sup>, SINGH MEWA<sup>3</sup>, SLIWA ALEXANDER<sup>4</sup>

<sup>1</sup>LTM-Research and Conservation, University of Mysore, Mysuru, Karnataka 570006, INDIA; niloferbegum3@gmail.com

<sup>2</sup>LTM-Research and Conservation, Eschenweg 5, 37130, Gleichen, GERMANY

<sup>3</sup>Biopsychology Laboratory, University of Mysore, Mysuru, Karnataka 570006, INDIA

<sup>4</sup>Zoologischer Garten Köln, Riehler Straße 173, 50735 Köln, GERMANY

The long-term survival of captive mammal populations is a key problem of conservation oriented zoo biology. They may not be functional as reserves; the conservation potential being low. Breeding problems are common: females may not breed at all or may not raise offspring successfully. This study is part of a comprehensive investigation on the development and conservation potential of the global captive lion-tailed macaque (Ltm) population with special reference to individual reproductive output. It covers 113 years, 2426 individuals in subpopulations and 324 institutions. It is based on studbooks and breeding programs' reports – covering periods prior to the latter's establishment (1899 - 1980) and the period until 2012. Aim of the study presented is to analyse the patterning of reproduction with reference to the population's productivity and its potential for long term survival.

The development of the global historical population in the first five decades reveals almost no breeding but the frequent introduction of wild caught individuals. In the next five decades, a moderate increase in population size via breeding followed. Since the 1990s the American subpopulation revealed a strong decrease due to birth control and breeding problems. The 2426 individuals of the historical population “resulted” in a total living population of 500 individuals in three subpopulations. However, the European population only with its 349 individuals in 2012 had a decent proportion of successfully breeding females. In the global historical population, a total of 1790 births with 1093 surviving offspring has been recorded for 438 females. About 60 % of the females and 80 % of the males in the global historical population did not contribute to the breeding pool. Even in the managed populations since the 1980s less than 50 % of the adult females reproduced successfully. Under these, few (24 %) produced 50 % of the offspring. About 50 % of the reproducing females produced 1-3 infants. Infant mortality oscillated around 30 % since the 1980s. More than 50 % of the 324 Ltm groups had less than six members over long time periods; 52 % remained with no or very little breeding; larger groups had a much higher number of births per female.

Large differences and unpredictability in reproductive output between the females characterize the global historical Ltm population. It is discussed that its demographic structures implicate life history patterns that are at risk to be maladaptive - decreasing its potential as a model and reserve for the wild population. Consequences for population and reproduction management are outlined.

## Resistance but low resilience to a virulent disease epidemic in a free-ranging population of spotted hyenas

BENHAIEM SARAH<sup>1\*</sup>, MARESCOT LUCILE<sup>1\*</sup>, EAST MARION L<sup>1</sup>, KRAMER- SCHADT STEPHANIE<sup>1</sup>, GIMENEZ OLIVIER<sup>2</sup>, LEBRETON JEAN-DOMINIQUE<sup>2\*\*</sup>, HOFER HERIBERT<sup>1\*\*</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Strasse 17, 10315 Berlin, GERMANY; benhaiem@izw-berlin.de

<sup>2</sup>CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier, EPHE, 1919 Route de Mende, 34293 Montpellier Cedex 5, FRANCE

\*joint first authors; \*\*joint last authors

As epidemics of infectious diseases can threaten endangered species, the development of mathematical tools that accurately predict their impact on wildlife populations is essential. Continuous time models, by far the most common type of epidemiological models, have substantially advanced our understanding of infectious diseases. However, the implications of such models remain at least partly theoretical because continuous-time models are challenging to combine with empirically estimated parameters. To bring more realism to this field, the use of discrete time models is increasingly encouraged. Using a data set collected during a period of two decades that includes more than 600 continuously monitored spotted hyenas (*Crocuta crocuta*), we quantified the impact on the dynamics of this population of a canine distemper virus (CDV) epidemic caused by a strain virulent to this species. We assigned female hyenas a demographic state, a social state and an infection state and developed a multi-event capture-mark-recapture model to estimate annual survival and transition probabilities between those states. These estimates were then used as input into a stage-structured population matrix model. During the epidemic, the population's growth rate  $\lambda$  was decreased by 9 %. The population regained its former size more than 15 years after the start of the epidemic. Our results reveal the essential demographic contribution to  $\lambda$  provided by socially dominant females. The basic reproduction number  $R_0$  of CDV increased from 0.9 during the pre-epidemic to 5.9 during the epidemic period. The hyena population had entirely rebuilt its pool of susceptible individuals 6 years after the end of the epidemic, indicating that the population is currently at risk if a virulent strain emerges again. By integrating detailed individual based empirical data with epidemiological concepts, we advance a new field of research that make it possible to study the interactions between epidemiological, demographic and social processes in wildlife populations, including those of endangered species.

## How to be sure of hedgehog absence before building projects, bush pruning or clearing events?

BERGER ANNE<sup>1,2</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Dept. of Evolutionary Ecology, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; berger@izw-berlin.de

<sup>2</sup>Wildlife Detection Dogs e.V., www.wildlifedetectiondogs.org, Sofienstraße 5, 74867 Neunkirchen, GERMANY

European hedgehogs (*Erinaceus europaeus*) are nocturnal, solitary, hibernating, ground-dwelling insectivores. Particularly in Great Britain, there is a threatening decline in the hedgehog abundance of which the causes are being speculated. In Germany, hedgehogs are strictly protected but population development is not known as monitoring would be very complex due to the hedgehogs' way of life.

According to the German federal law on nature protection (BNatSchG § 42), it is not allowed to remove, damage or destroy the daytime or the winter nests of hedgehogs. These nests are mostly hidden in bushes close to meadows but they cannot be discovered by simple visual inspections of the bushes. Hence, nightly spotlighting was described as the most effective method to detect hedgehogs. However, nocturnal spotlighting is highly time-consuming and unreliable for consultancy or planning offices. Thus, hedgehogs are mostly not considered during local building or planning work. Moreover, out of respect for breeding birds and by law, most pruning or wood clearing work is done during the hibernation period of the hedgehog (November until April) in which these animals are unable to escape or move.

Wildlife detection dogs (trained on hedgehog odour) can be used to detect and indicate the hidden hedgehog nests. The dogs can work during the day in both summer and winter. As the hedgehogs stay in their nests at these times, a systematic search is possible. When indicating the nests, the dogs should not touch it in order to not disturb the animals in the nest. Hedgehog detecting dogs are now used in Zurich, the Lucerne region (Switzerland) and in Berlin (Germany). Considering the decline in the hedgehog abundance, hedgehog detecting dogs are recommended to be used as standard method in consultancy or planning offices during local planning and building processes. Using detection dogs will greatly improve the feasibility of removing the hedgehogs temporarily from the danger zone to protect the animals.

## Habitat influence on haematological parameters of red deer (*Cervus elaphus*)

BIANCHI ALESSANDRO, GIACOMELLI STEFANO, ARCHETTI IVONNE, ROTA NODARI SARA

Istituto Zooprofilattico Lombardia ed Emilia Romagna “Bruno Ubertini”, Via Bianchi 9, 20124, Brescia, ITALY; alessandro.bianchi@izsler.it

The relation between haematological parameters and habitat type was investigated in an alpine population of red deer. The study was carried out on 202 red deer culled for a population management control plan in the period from 31 October 2014 to 28 January 2015 in a selected area (about 25.100 ha) of the Stelvio National Park.

The area was divided into ten smaller zones with different environmental characteristics, classified through geolocalisation, Corine Land Cover 2006 (CLC). Demographic and habitat use data was gathered by census and GPS collar data recorded by the park institution.

Haematological parameters (HP) (total protein, urea, creatinine, albumin, globulin, A/G ratio, haptoglobin, haemolytic complement, lysozyme, cortisol and protein electrophoresis) were tested on 61 suitable samples.

According to culling records and census data five higher density zones (over 13 deer/100 ha estimated) were identified, representing approximately one third of the study area. These zones were located at the centre of the study area, characterized by a greater presence and continuity of CLC category “permanent pastures” and were massively grazed by domestic livestock.

Female deer culled in these zones were older than in the others (Mann-Whitney,  $p = 0.005$ ) and their prevalence of pregnancies (presence of *corpus luteum*) were significantly lower ( $\chi^2$ ,  $p = 0.05$ ). This last evidence could validate the hypothesis of higher density. Moreover, their home ranges, recorded by the park staff showed also a behaviour mostly non-migratory.

The comparison of the HP between the animals in the high density area and the others suggested different body conditions and immunological status.

In particular, adults (> 1 year old) living in higher density zones had higher total protein (ANOVA,  $p = 0.003$ ), gamma globulin (ANOVA,  $p < 0.001$ ), urea (ANOVA,  $p = 0.003$ ) and a lower A/G ratio (ANOVA,  $p = 0.002$ ). No significative differences were found on young subjects (< 1 year old).

These results demonstrate the influence of good pasture on HP. In particular protein and urea concentration were closely related to pasture’s quality as previously described. The simultaneous increment of gamma globulin could be interpreted as a major allocation of energy in immunocompetence (antibodies) or as an effect of the higher density. More probably this could be a result of the homeostatic trade-off between higher trophic availability and higher inter-specific competition, represented also by higher pathogen exchanges.

Our results could be helpful to develop an integrated approach to population management and epidemiological surveillance.

## Occupancy, local abundance and density: what can we estimate with camera-trapping data

BIN MOHAMED AZLAN<sup>1</sup>, SOLLMANN RAHEL<sup>2</sup>, WONG SETH TIMOTHY<sup>3</sup>, ABRAMS JESSE FRANK<sup>1</sup>, WILTING ANDREAS<sup>1</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; mohamed@izw-berlin.de

<sup>2</sup>Department of Wildlife, Fish and Conservation Biology, University of California Davis, Davis, 95616 California, USA

<sup>3</sup>Carnivore Ecology Laboratory, Forest and Wildlife Research Center, Mississippi State University, Mississippi State, 39759 Mississippi, USA

Abundance and density are important measures of the state of wildlife populations and fundamental to evaluate the effectiveness of conservation programmes or the sustainability of forest management. However, abundance and density are not easy to estimate for many wildlife species, particularly for elusive species occurring in tropical rainforests. In such environments, research mainly relies on remotely operating camera-traps to survey wildlife populations. For individually identifiable species, which includes many cat species, camera-trap data allow researchers to estimate density using modern spatial capture recapture (SCR) models. However, for most species distinguishing individuals is difficult. In such cases, researchers rely on species level detection data in combination with occupancy models to assess habitat associations and species distributions. Although in some cases abundance and distribution of species are linked, previous studies have demonstrated that occupancy does not always represent abundance. A compromise between occupancy and SCR models are N-mixture models, which only require identification of individuals within one station during one occasion to estimate local abundance. Here, we tested the relationship between occupancy, local abundance (N-mixture models), and density (SCR models) with a camera-trap dataset for two small carnivore species; the Malay civet (*Viverra zibetha*) and the banded civet (*Hemigalus derbyanus*). Camera-trap data was collected in a two-phase design (coarse grid for occupancy and local abundance and fine grid for density estimation) from two commercial forest reserves under different forest management strategies in Sabah, Malaysian Borneo. The relationships between the three measures may be species- and even site-specific. However, by assessing the relationship among the three measures in our study sites, we provide information that allows conservationist and forest managers in Sabah to better determine the optimal measure to be used for their given conservation targets.

## **Genetic diversity and population structure of *Angiostrongylus vasorum* parasites in urban foxes (*Vulpes vulpes*) of London, UK**

BLANCH-LÁZARO BERTA, BLAKE DAMER

University of London, The Royal Veterinary College, Department of Pathobiology and Population Sciences, Hawkshead Lane, North Mymms, AL9 7TA, Hatfield, UK; bblanch6@rvc.ac.uk

*Angiostrongylus vasorum* is a nematode parasite of the pulmonary arteries and the heart that infects domestic and wild canids, with dogs (*Canis familiaris*) and red foxes (*Vulpes vulpes*) as the most commonly affected definitive hosts. Whereas dog health can be severely compromised, the actual consequences of infection on fox health are not fully understood. Disease occurrence has been reported to be limited to endemic foci throughout Europe. Distribution in Great Britain is not uniform, with two hotspots occurring in South England and Wales. Recent studies suggest that angiostrongylosis is an emerging disease, and that red foxes may play an important role in the epidemiology of the parasite. Genetic analysis done in parasites collected from dogs and foxes throughout Europe have shown that the same parasite haplotypes are commonly shared between different host species. However, no studies have looked at *A. vasorum* genetic diversity within a small geographic area, or within individual hosts. The objective of the present study was to assess the genetic diversity of *A. vasorum* a) hosted in foxes within the Greater London area (single worm per fox dataset); and b) hosted within single foxes (multiple worms per fox dataset). During 2016, *A. vasorum* worms were collected from foxes culled in London. Subsequently, DNA was extracted and partial fragment of the cytochrome oxidase subunit 1 (COI) mitochondrial gene was amplified and sequenced. Sequences from the single worm dataset were compared to those published by JEFFERIES *et al.*, 2010. Multiple haplotypes (n = 19) were described, 15 of them were identified from foxes found in London. This suggests that despite London being a relatively small geographic area, large genetic variation can be found. On the multiple worm dataset, multiple haplotypes (n = 22) were described from multiple worms from six different foxes. This is the first study to confirm that different haplotypes can be found infecting a single fox at a specific time, emphasising their relevance to the potential for genetic exchange between *A. vasorum* in the UK.

JEFFERIES R, SHAW SE, WILLESEN J, VINEY ME, MORGAN ER (2010): Elucidating the spread of the emerging canid nematode *Angiostrongylus vasorum* between Palaearctic and Nearctic ecozones. *Infect Genet Evol* **10**, 561–568.

## Communicative behaviour of Amur tiger and Amur leopard: analysis of scent-marking activity in wildlife

BLIDCHENKO EKATERINA Y<sup>1</sup>, SHEVTSOVA ELENA I<sup>1</sup>, MATYUKHINA DINA S<sup>1</sup>, YACHMENNKOVA ANNA A<sup>2</sup>, VITKALOVA ANNA V<sup>1</sup>, SONIN PYOTR L<sup>1</sup>, SEDASH GLEB A<sup>1</sup>, STOROZHUK VIKTOR B<sup>1</sup>

<sup>1</sup>United Administration of the State Nature Biosphere Reserve “Kedrovaya Pad” and “Land of the Leopard” National Park; pr. 100-letiya Vladivostoka 127, Primorskiy kray, Vladivostok, 690068, RUSSIA; avulpes@yandex.ru

<sup>2</sup>Severtsov Institute of Ecology and Evolution, Russian Academy of Science, Leninsky pr. 33, Moscow, 117071, RUSSIA;

The south-west of the Russian Primorsky region and borderline areas of China is the only area in the world inhabited by two northernmost species of *Panthera* genus – Amur tiger (*Panthera tigris altaica*) and Amur leopard (*Panthera pardus orientalis*) which are included in IUCN Red List (ver. 3.1) as critically endangered and endangered, respectively. We characterized the mutual peculiarities of scent-marking behaviour of the two species inhabiting the south-west of Primorsky region of the Russian Far East. Period of data collecting is 2015 to 2017 in the territory of “Land of the Leopard” National Park (2800 km<sup>2</sup>). To detect animals in the wild we used camera traps (ScoutGuard, Bestok, Bushnell) programmed to video recording mode from 10 to 30 sec. We established trail cameras at places with increased scent-marking activity of tigers and leopards. We have identified five types of attractors: crag, cliff, trail, tree, and substrate. Totally, the data were collected from 9 scent marking points, 41 scent marking acts were recorded, and 11 categories of behaviour were identified. To analyse the behaviour, an “ethogram” with 14 types of activity was created; visits of all other animals to scent-marking points were also registered.

Tiger and leopards were recorded at the same scent-marking points. In addition to them animals of 12 other species were also registered. Leopards differ from tigers in their attractor preferences; they tend to use crags (t-test;  $p = 0.03$ ), while tigers more frequently choose for scent-marking trees and trails (t-test;  $p = 0.08$ ). The leopards’ choice of attractors statistically depends on the season (GLM;  $SS = 2759.5$ ;  $F = 2.82$ ;  $p = 0.044$ ), while influence of season on the intensity of scent-marking in tigers was not detected. At the same time, the intensity and frequency of tigers’ scent-marking significantly depends on  $\sum$  combination of factors – a tree located on a trail (ANOVA  $p = 0.00$ ). For all animals recorded in the scent-marking points, the results show absence of any significant differences, association of scent-marking activity to any particular time of the day. The peak of scent-marking activity for all animals are winter and spring seasons. The most appealing attractor for all animals (regardless of species) is crag – 42 % ( $7.03 \pm 10$ ; mean  $\pm$  SD). This parameter significantly differs from trail ( $2.2 \pm 2.6$ ;  $Z = -4.44$ ;  $p = 0.00$ ), tree ( $2.8 \pm 2.2$ ;  $Z = 2.78$ ;  $p = 0.00$ ) and substrate ( $2.2 \pm 1.3$ ;  $Z = 3.11$ ;  $p = 0.00$ ) used by animals (Mann-Whitney U Test).

## Day to day fluctuations in okapi weight – a signal of problem?

BRANDLOVÁ KAROLÍNA<sup>1,2</sup>, GAŠPAROVÁ KATEŘINA<sup>1</sup>, ČULÍK LUDĚK<sup>2</sup>

<sup>1</sup>Czech University of Life Sciences Prague, Department of Animal Science and Food Processing in Tropics, Kamýcká 129, 165 00, Praha 6, CZECH REPUBLIC; karolina@derbianus.cz

<sup>2</sup>Dvůr Králové Zoo, Štefánikova 1029, 544 01 Dvůr Králové nad Labem, CZECH REPUBLIC

Okapi (*Okapia johnstoni*), the shy forest giraffe, still represents a challenge for zoo breeders in terms of husbandry, health care, and nutrition. Changes in body weight may reveal potential problems and regular weight monitoring is therefore applied in most of the breeding facilities. We aimed to analyse data from long-term weighing of five individuals (three males, two females) kept in Dvůr Králové Zoo. The data were collected from 2004 to 2017, in total 6440 weight records. In this study we focused on day to day fluctuations in the body weight, i.e. gains and losses. The mean day fluctuation differed according to the age category and was the highest in calves ( $0.35 \pm 1.59$  kg,  $0.3 \pm 1.1$  % of body weight) and the lowest in adults ( $0.015 \pm 2.27$  kg,  $0.001 \pm 0.8$  % of body weight, ranging from -16.5 to 18 kg, from -7 to 7 % of body weight).

The birth weight of two calves born during the study period was 28.5 kg (male) and 25.5 kg (female). The weight of both suckling calves continually increased until the age of 61 and 21 days, respectively, when the first day to day weight losses appeared. Weight losses larger than 2 % appeared regularly since the calves reached the age of 3 months in the male calf and as late as at 7 months in female calf. The highest weight loss recorded in suckling calf was 4 % of body weight (5.5 kg) in 7 month old male calf.

The adult okapi is expected to consume the amount of food of about 1.8 % of its body weight. The day to day weight fluctuations may incidentally reach up to 7 % of body weight (more than 15 kg). However, the fluctuations higher than 3 % of body weight formed only 1 % of all records. On the contrary, the periods with weight losses reported continually for more than five days in one individual were connected to stress factors, specifically to the reconstruction of the roof of the stable.

## Foraging habitat utilisation map for Lyle's flying fox (*Pteropus lylei*) by high-resolution GPS tracking in Central Thailand

CHAIYES AINGORN<sup>1,2</sup>, DUENGKAE PRATEEP<sup>1</sup>, PONGPATTANANURAK NANTACHAI<sup>1</sup>,  
WACHARAPLUESADEE SUPAPORN<sup>3</sup>, HEMACHUDHA THIRAVAT<sup>3</sup>

<sup>1</sup>Kasetsart University, Department of forest biology Faculty of Forestry, Ngam Wong Wan Rd. 10900 Bangkok, THAILAND; chaiyes.stou@gmail.com

<sup>2</sup>Center for Advanced Studies in Tropical Natural Resources, Kasetsart University, Ngam Wong Wan Rd. 10900 Bangkok, THAILAND

<sup>3</sup>Chulalongkorn University, World Health Organization Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Phayathai Rd. 10330 Bangkok, THAILAND

Flying foxes (*Pteropus* spp.) play a key role in forest regeneration as seed dispersers. Flying fox populations have been shrinking in recent years due to poaching and habitat loss. Moreover, little is known about their foraging habitat, particularly in Southeast Asia. Studies on foraging-habitat relationships in the context of these changes have been very few, and the lack of information hinders management efforts. Lyle's flying fox (LFF) (*Pteropus lylei*) are declared as Vulnerable on the IUCN Red List due to a substantial population decline. Accurate assessments of the factors that influence LFF foraging habitat are needed in Thailand as management tools to reduce human-wildlife conflicts. There, the biggest LFF populations are restricted to a few core areas embedded in central Thailand. This system will provide accurate baseline data for more effective management. However, identifying suitable foraging sites of LFF in the entirety of Thailand is very costly and time-consuming. Therefore, an alternative is to conduct presence-only surveys which predict the proportion of sites utilized by a species using spatial distribution over a large landscape. We used ecological niche modelling to identify the key factors that influence foraging habitat utilisation of LFF populations. Maximum entropy (MaxEnt) modelling technique was used to model 30 meters resolution environmental factors: 17 land cover types including LFF's food types, elevation, precipitation, and distance to main streams. The total of 348 presence points were generated from 19 individuals LFF's using high-resolution global positioning system (GPS) loggers. These data points were then ground truth checked as actual foraging point data. Model performance was evaluated by area under the curve (AUC). The spatial foraging utilisation model of LFF was predicted as excellent using  $AUC > 0.9$ . The results indicate that elevation, annual precipitation, and land cover were the most influential for predicting a potential foraging habitat utilisation of LFF. This study provides an initial understanding on how the foraging area of LFF will be influenced by flat plain, land cover types (mixed orchard, mango and water body) and annual precipitation (90 - 115 mm/month). Moreover, the highly suitability of foraging utilisation of LFF were overlapped with cultivated fruit areas, which could potentially lead to conflicts with local farmers. Our study suggests that to reduce the potential for conflicts between bats and humans on crops, the landscape management plan should preserve and increase remaining natural fruit trees in the study area as resources for LFF.

## **Behavioural interactions of sloth bears with special reference to human-sloth bear conflict and management implications in Central India**

CHAUHAN NETRAPAL SINGH

Amity Institute of Wildlife Sciences, Amity University Uttar Pradesh, Noida – 201 303, Gautam Buddha Nagar U.P., INDIA; nschauhan@amity.edu

In Bilaspur Forest Division, Madhya Pradesh, Central India, human casualties and agricultural crop damage by sloth bear (*Melursus ursinus*) are serious problems. Human and bears live together and share few common resources. People benefit themselves in such situation, disturbing bears. As a result, attacks on human beings by sloth bears are on the increase. We studied the behavioural interactions of sloth bears, human-sloth bear conflict and management implications. Based on information of the forest department, village interview and analysis of human casualties, the conflict problems have been evaluated. During 2000 to 2010, 393 human mauling and killing cases occurred; most cases occurred in forests, followed by crop fields and villages. Attacks were more on males (68.6 %) and less on females (31.4 %). There was marked monthly variation in human casualties. Maximum incidences occurred during monsoon season. The victims were found involved in cattle grazing (24.9 %), farming or crop protection (11.7 %), defecation (28.8 %) or moving in forests (22.6 %) or vicinity of villages (3.3 %) or non-timber forest produce collection (8.7 %). The victims were mainly in the age group of 19-30 years (30.4 %) and 41-50 years (20.6 %). Most of these incidences occurred between 0400 to 1400h. Fifty eight cases (56.9 %) occurred between 0400 to 1000h when bears remained most active, and 21 cases (20.6 %) occurred between 1000-1400h when bears were comparatively less active. In most of the cases, bears were found to attack running on its hind and fore legs and pushing the victims (36.5 %). Whereas, bear attacked 28.47 % victims standing on its hind legs, and 10.22 % of victims were fell on the ground, 5.11 % were sitting and 1.46 % climbed on trees at the time of attack. There were 56 casualties (54.9 %) caused by single bears; 30 cases (29.4 %) occurred when mother and cub(s) were together, and 14 cases (13.7 %) when bears were in group of 2 or 3. Three persons were killed and one male and two females were killed and eaten. In 2.19 % cases, victims became unconscious or died on the spot. There were either multiple injuries to body of the victims (51.82 %), or injuries to legs (24.82 %), or hands (12.41 %) or chest and head (8.03 %). In 4 cases (2.92 %), injuries were on abdominal and hip regions. Competition for resource sharing, non-timber forest produce collection, human disturbances, livestock grazing, feeding on crops and farming or crop protection were found to be the important factors responsible for rise in human-sloth bear conflicts. Strategies for mitigation human-sloth bear conflicts are suggested.

## **Positive effects of biodiversity – Why edible dormice do not stick with their preferred food plant**

CORNILS JESSICA SVEA; HOELZL FRANZ; ROTTER BIRGIT; BIEBER CLAUDIA; RUF THOMAS

University of Veterinary Medicine Vienna, Department of Integrative Biology and Evolution, Savoyenstraße 1, 1160, Vienna, AUSTRIA, jessica.cornils@vetmeduni.ac.at

One strategy of tree species to maximize offspring survival is the large-scale synchronization of their seed production, a so called seed mast event. This can lead to a major resource pulse that has an immense influence on other species, which use this increased food availability to maximize their reproductive output too. Among other species edible dormice are highly dependent on these pulses of energy rich seeds (e.g. *Fagus sylvatica*) to reproduce. It is especially important for juveniles, which need the seeds that are high in fat content, to gain enough mass prior to hibernation. We therefore hypothesized that edible dormice either switch territories and move to areas with alternative foraging opportunities in years without mast, or choose a habitat where alternative food sources are given, but which are less optimal in mast years. We analysed the habitat preferences of edible dormice with environmental niche factor analyses (ENFA) for nine years of capture-recapture data in the Vienna Woods (Lower Austria, 48°05'N/15°54'E; 400–600 m asl).

Our results showed that the structure of the forest, especially vertical stratification and canopy closure played major roles in the habitat choice of the animals. This hints towards a predator avoidance strategy, because owls (one of the main predators of these rodents) cannot manoeuvre well in dense forest. Furthermore, we found that dormice avoided areas with higher beech tree density and preferred sites with a relatively high proportion of coniferous trees. In these areas, food sources like conifer cones and leaves are also available in non-mast years and are less variable in abundance than beech seeds.

Our results give therefore a strong evidence for positive effects of biodiversity. Edible dormice prefer areas with diverse tree species to optimize survival and reproduction under changing food conditions.

## **Troublesome neighbours – the stressful life of a small rodent in European forests**

CORNILS JESSICA SVEA<sup>1</sup>, HOELZL FRANZ<sup>1</sup>, HUBER NIKOLAUS<sup>1</sup>, BIEBER CLAUDIA<sup>1</sup>, SCHWARZENBERGER FRANZ<sup>2</sup>, RUF THOMAS<sup>1</sup>

<sup>1</sup>University of Veterinary Medicine Vienna, Department of Integrative Biology and Evolution, Savoyenstraße 1, 1160, Vienna, AUSTRIA, jessica.cornils@vetmeduni.ac.at

<sup>2</sup>University of Veterinary Medicine Vienna, Institute for Medical Biochemistry, Department for Biomedical Sciences., Veterinärplatz 1, 1210, Vienna, AUSTRIA

The presence of predators changes the behavioural and physiological condition of their prey. These changes can be measured either via visual markers or by monitoring physiological parameters such as glucocorticoid hormone production. Glucocorticoids are secreted within minutes during a stressful event and are suggested to play an important role in re-establishing homeostasis during life threatening situations. In the field, measuring hormone levels can be challenging, especially when trying to minimize the impact on the animals. Faecal cortisol metabolites (FCM), the metabolites of glucocorticoids in the faeces, can be repeatedly and noninvasively measured from the same individual. A further advantage is that the elevation of hormone levels is delayed in the faeces and can be measured during the subsequent day, which excludes a handling bias in the sample. We assessed the influence of predation pressure (i.e. owl density as measured by breeding owl pairs) on the Hypothalamic-pituitary-adrenal (HPA) axis of edible dormice. This species is highly bound to energy rich seeds of beech trees and can only reproduce if these seeds are available in mast years. In years without mast, we expected the animals to forage longer, to gain the same amount of body mass, leading to a higher predation risk. We investigated free living dormice in the Vienna Woods and performed a supplementary feeding experiment to answer the following questions: Is a higher predation pressure associated with elevated glucocorticoid levels? Does low caloric food in non-mast years result in higher glucocorticoid levels? And finally, does the chronic stress hypothesis, which states that glucocorticoids are downregulating reproduction, apply in this population? After performing a biological validation of the FCM assays, we analysed 104 samples of female edible dormice. The animals had significantly higher FCM levels when their nest-box was situated in an area within a tawny owl territory. There was no significant difference between animals in non-mast vs. mast years. Animals that were supplementary fed on the other hand, had higher levels of glucocorticoids. This could however, be related to a territorial effect, with animals defending the nest-box with the supplementary food against intruders. There was no negative effect of glucocorticoid hormones on reproduction. Together our results indicate that glucocorticoid hormone regulation has an important influence on predator tolerance for edible dormice but plays only a minor role in dealing with seasonal variation and reproduction.

## **Captive breeding: Additional considerations for studbook breeding recommendations**

COWL VERONICA<sup>1,2</sup>, WALKER SUE<sup>1,2</sup>

<sup>1</sup>EGZAC, Chester Zoo, Caughall Road, Upton-by-Chester CH2 1LH, UK; v.cowl@chesterzoo.org

<sup>2</sup>Chester Zoo, Caughall Road, Upton-by-Chester CH2 1LH, UK

For many endangered species the results of *in situ* conservation efforts alone would not occur fast enough to prevent extinction therefore, their survival is increasingly more reliant on *ex situ* conservation methods. Zoological institutions often participate in captive breeding; an *ex situ* approach to maintain healthy breeding populations with the intention of reintroduction once threats in their natural habitat have been removed. Breeding recommendations are made in which individuals are carefully selected by their pedigree to prevent inbreeding and to ensure that genetic diversity is maintained. Currently however, the relative success of these breeding recommendations is unknown, with a 2011 study on AZA breeding programme recommendation outcomes finding success in only 22 % of recommendations. While low rates of success are attributable to multiple factors including logistics, illness and death, or institutional activity, subfertility of the breeding individuals also plays a role. Using a series of case studies of Eastern black rhino, Komodo dragons, and Sulawesi crested macaques from Chester Zoo and EGZAC, we illustrate that there is a need to assess intermediate individual, group and population level factors preventing the growth of healthy and sustainable captive populations. Using a combination of endocrinology, nutrition, ethology and husbandry surveys we suggest a series of practical tools that collections and breeding programmes can implement to improve the success of breeding programmes and the welfare of the animals within them.

## **Insight into predation behaviour of tiger in Ranthambore Tiger Reserve, Rajasthan, India: Use of low cost gel-based molecular sexing of prey hairs from scats**

DE RAHUL<sup>1</sup>, JOSHI BHEEM DUTT<sup>1</sup>, SHUKLA MALAY<sup>2</sup>, PANDEY PUNEET<sup>3</sup>, SINGH RANDEEP<sup>3</sup>, GOYAL SURENDRA PRAKASH<sup>1</sup>

<sup>1</sup>Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand, PIN – 248001, INDIA; goyalsp@wii.gov.in

<sup>2</sup>Gujarat Forensic Sciences University, Gandhinagar, Gujarat, PIN – 382007, INDIA

<sup>3</sup>Amity Institute of Wildlife Sciences, Amity University Campus, Sector-125, Gautam Buddha Nagar, Noida, Uttar Pradesh, PIN – 201303, INDIA

Elucidating ecology of the species and prey-predator relationship in different bioclimatic regions through diet reconstruction is of utmost importance for planning effective conservation strategies for long ranging carnivores like the tiger. The literature indicates that cervid males are more prone to predation, possibly shaping the demography of the prey species. Micro-histological analyses of undigested prey hairs from tiger scats have widely been used for reconstruction of prey composition. However, sex of the prey is usually determined by recorded kills. Despite extensive and intensive monitoring, the number of such records remains low. The collection of scats from the field incurs relatively lower effort and cost in comparison to recording kills. Here we describe a gel-based molecular sexing method using DNA yielded from a single prey hair extracted from tiger scat after prey species identification through microscopy. We extracted nuclear DNA from the root part of prey species' hair isolated from confirmed tiger scats (n = 42) collected from Ranthambore Tiger Reserve, Rajasthan, India. We validated the absence of tiger DNA using felid-specific microsatellite loci. We found that up to 230 bp long nuclear and mitochondrial fragments could be amplified successfully from DNA extracted from a single prey hair. We amplified a Y-linked fragment of 224 bp for identifying male individuals while using a mitochondrial marker of 151 bp linked to the 12S rRNA gene as an internal control for PCR amplification. We affirmed the results of the molecular sexing by amplifying X and Y specific alleles of the cervid amelogenin gene in a subset of the samples (n = 10). Sambar (*Rusa unicolor*) and chital (*Axis axis*) were the principal prey of tiger in the study area with frequencies of occurrences of 47.6 % and 30.9 %, respectively in scats. The proportions of males of all sambar and chital prey in tiger diet were 80 % and 61.5 %, respectively. Male-biased predation was statistically significant in sambar (p < 0.05). The results corroborated the literature reporting low proportion of males documented in wild populations. This technique identifies the sex of young animals, which is difficult to ascertain from kills. Therefore, the described method and its application have the potential to augment information on the diet of the tiger as well as other carnivores along with underlying prey-predator dynamics to a finer resolution.

## **Faeces and urine matter! Non-invasive monitoring of stress and health in wild primates**

DEFOLIE CHARLOTTE<sup>1,2</sup>, KRAUS CORNELIA<sup>1</sup>, FICHEL CLAUDIA<sup>2</sup>, HEISTERMANN MICHAEL<sup>3</sup>

<sup>1</sup>Sociobiology-Anthropology Department, University of Göttingen, Kellnerweg 6, 37077, Göttingen, GERMANY

<sup>2</sup>Behavioral Ecology and Sociobiology Unit, German Primate Center (DPZ), Kellnerweg 4, 37077, Göttingen, GERMANY; cdefolie@dpz.eu

<sup>3</sup>Endocrinology Laboratory, German Primate Center (DPZ), Kellnerweg 4, 37077, Göttingen, GERMANY

As infectious diseases are putting more pressure on declining animal population, and zoonotic outbreaks are increasing in frequency and severity, stress and health monitoring in wildlife seems crucial for both conservation plans and human health management. However, ethical and practical difficulties for health monitoring of wild animals are more the rule than the exception. Here, we present a few technics specifically developed or used within the Research Group “Sociality and Health in Primates” funded by the “Deutsche Forschungsgemeinschaft (DFG)”. We focused on non-invasive technics requiring little to no cooperation of the animals, using saliva, urine or faeces to measure short-term and long-term physiological stress levels, inflammation reaction, immune system activation, nutritional status and parasites and pathogens in six projects with different primate species. We show that non-invasive monitoring of stress and health in wildlife can be easily conducted in field conditions, with regular collection of products usually considered as a waste and without stress or disturbance of the animal behaviour and physiology. We believe that these methods can allow a better monitoring of endangered animal population and zoonotic outbreaks with an enhanced understanding of the sociality-stress-health relationship in group living animals.

## **So happy together? Ecological and social correlates of stress in wild redfronted lemurs (*Eulemur rufifrons*)**

DEFOLIE CHARLOTTE<sup>1,2</sup>, HEISTERMANN MICHAEL<sup>3</sup>, FICHTEL CLAUDIA<sup>2</sup>, KRAUS CORNELIA<sup>1</sup>

<sup>1</sup>Sociobiology-Anthropology Department, University of Göttingen, Kellnerweg 4, 37077, Göttingen, GERMANY

<sup>2</sup>Behavioral Ecology and Sociobiology Unit, German Primate Center (DPZ), Kellnerweg 6, 37077, Göttingen, GERMANY; cdefolie@dpz.eu

<sup>3</sup>Endocrinology Laboratory, German Primate Center (DPZ), Kellnerweg 6, 37077, Göttingen, GERMANY

Group living provides benefits such as reduction of predation risk and joint resource defence but also bears costs such as increased risk of pathogen transmission. In particular, social organization and social relationships can have a profound impact on the physiological stress response, which in turn plays a pivotal role in mediating the link between sociality and health. On the one hand, sociality can be a source of stress for individuals, as they have to deal with dominance and unpredictable changes in their social network. On the other hand, social support and strong social bonds can reduce the adverse effects of stress and improve an individual's health and fitness. We investigated ecological and social correlates of physiological stress in redfronted lemurs (*Eulemur rufifrons*) in Kirindy forest, Western Madagascar. Their social system deviates from that of the better-studied anthropoid species, with high levels of affiliation and social tolerance but also quite regular group takeovers and evictions upsetting social stability. We observed 32 adult individuals in five groups over a period of 18 months and collected regular fecal samples for glucocorticoid metabolite (fGCM) analyses. We focused on the influence of quality and quantity of social relationships and sex differences on fGCM concentrations. Preliminary results indicate that the dry season is characterized by elevated fGCM levels in all individuals. Reproductive season also impacted on fGCM, with increased fGCM concentrations found in females during gestation and early lactation, and during the mating season and the early lactation period in males. Finally, social instability was positively correlated with fGCM, while strong social bonds showed a negative correlation with fGCM levels in both sexes, supporting the social buffering hypothesis in this species.

## **Resource partitioning between spotted hyenas (*Crocuta crocuta*) and lions (*Panthera leo*)**

DHEER ARJUN

Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; arjdheer@gmail.com

The negative impact of anthropogenic activities on wildlife has led to the establishment of protected areas to reduce human-wildlife conflict. These protected areas are often fenced in order to meet the needs of expanding human communities and to conserve declining wildlife populations. This creates challenges for the management of wide-ranging animals such as large carnivores, especially those that compete with one another for limited resources. This study focused on resource partitioning between GPS-GSM collared spotted hyenas and lions in Lewa Wildlife Conservancy and the adjacent Borana Conservancy in Kenya. Scat analysis revealed that spotted hyenas and lions showed a high degree of dietary overlap, though spotted hyenas had broader diets and fed also on livestock species, which lions mostly avoided. Spatially, spotted hyenas showed stronger intraspecific avoidance and more exclusive territorial behaviour than lions did. Spotted hyenas and lions had a high degree of spatial overlap, and lions may have influenced den site selection in spotted hyenas. Both species were strongly nocturnal and crepuscular, with spotted hyenas travelling significantly further at night than lions did. Spotted hyenas and lions both displayed mixed results in dynamic spatiotemporal interactions, with 40 % of spotted hyena-lion pairs showing attraction and 70 % of pairs showing simultaneous use of overlapping areas. All but one inter-clan spotted hyena pairs showed strong avoidance, though lion pairs were not as mutually repulsive. This might indicate that scavenging and kleptoparasitism likely play a role in their dynamic. Based on data from this study and long-term predator monitoring at these conservancies, the spotted hyena population is suggested to be growing and healthy, though the lion population is of concern due to lower density, isolation, and low recruitment. Further investigation of human-carnivore conflicts within surrounding communities, long-term demographic trends of all members of the large carnivore guild, and the potential development of a dispersal-based metapopulation management scheme will allow for the continued persistence of large carnivores in the Lewa-Borana Landscape (LBL) and their coexistence with human communities.

## **Mountain goat glucocorticoids: correlates with endogenous, exogenous and fitness components in the wild**

DULUDE-DE BROIN FRÉDÉRIC<sup>1</sup>, MASTROMONACO GABRIELA F<sup>2</sup>; CÔTÉ STEEVE D<sup>1</sup>

<sup>1</sup>Université Laval, Département de biologie and Centre d'études nordiques, 2325 Rue de l'Université, G1V 0A6, Quebec, Québec, CANADA; frederic.ddb@gmail.com

<sup>2</sup>Toronto Zoo, Reproductive Physiology, 361A Old Finch Avenue, M1B 5K7, Toronto, Ontario, CANADA

Monitoring glucocorticoids can be useful to identify the challenges faced by wild animal populations and the mechanisms driving life-history strategies. While some experimental manipulations of glucocorticoid levels in captivity yielded compelling evidence supporting the use of this biomarker as a relevant conservation tool, the high number of confounding variables influencing hormone secretion makes it hard to observe consistent responses in natural environments. Long term studies accounting for age, sex, individual variation, life history stage and ecological context are needed to explore the limits of this method and to better understand the role of glucocorticoids in natural conditions. Using 600 hair and 800 faecal samples collected over 15 years from a free-living population of mountain goats (*Oreamnos americanus*) in Canada, we assessed the influence of endogenous (age, sex, mass, nutritional status, social rank and reproductive status) and exogenous (density, predation and resource availability) factors on baseline glucocorticoids levels. We then evaluated whether baseline levels were linked to fitness components such as reproductive success and survival among individuals. Low faecal crude protein concentration (i.e. an indicator of diet quality) and large body mass were related to low GC concentrations in faecal and hair samples, respectively. Among exogenous factors, high predator occurrence and late peak of resource availability were related to elevated GC concentrations in both tissues. Although these results strengthened the use of glucocorticoids in ecological studies, we found high temporal and individual variability even after accounting for multiple individual and environmental variables. Our study supports the idea that while useful to understand the mechanisms underlying life-history trade-offs, caution is required when using glucocorticoids for conservation or monitoring purposes. Extensive background information on the study system seems essential to adequately interpret GC measurements.

## Self-pressurized vitrification of feline oocytes

FERNANDEZ-GONZALEZ LORENA, JEWGENOW KATARINA

Leibniz Institute for Zoo and Wildlife Research, Dept. Reproduction Biology, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; fernandez@izw-berlin.de

Cryobanking is outlined as the best option to preserve genetic diversity in extremely threatened populations. Among all the cells that can be frozen, gamete cryopreservation represents the easiest way to guarantee the maintenance of genetic heterogeneity into the living populations. Even though felids are one of the most endangered families, methods for preserving germinal cells are still not reliable. Vitrification of oocytes shows insufficient survival rates after warming when compared with other species. Specific characteristics of the cat oocyte might need appropriated adaptations of the methods to obtain successful results. Recently, self-pressurized rapid freezing of different cell lines by using metal tubes has been described, showing an instantly cryofixation (GRABENBAUER *et al.*, 2014).

We hypothesized that, using this procedure for vitrification of feline oocytes might improve the outcome considering that no ice crystal can be developed into the sample, due to the internal pressure. Also, the high conductivity of the material compared to standard plastic devices would give a higher cooling and warming rate. Therefore, the aim of this study was to check whether the use of high pressure freezing methodology could increase the survival of vitrified feline oocytes. After retrieval by slicing the outer surface of domestic cat ovaries, batches of two to three oocytes were frozen directly at immature stage, with our 3-step method for vitrification (MIKOLAJEWSKA *et al.*, 2012) and were placed into aluminium tubes (outer diameter 0.6 mm/ inner diameter 0.3 mm), closed both sides with pliers and plunged into liquid nitrogen. Against our expectations the concentration of cryoprotectants used could not be reduced, as the use of a final concentration of 20 % showed no polar body after *in vitro* maturation. When using the regular concentration of 40 %, 22.9 % of the oocytes could be successfully matured after warming and after consequent fertilisation by ICSI, cleavage rate was 15.4 %. This data is lower than in previous studies.

For toxicity evaluation, oocytes and sperm cells were incubated in the tubes without freezing. Harmful effects were determined when cryoprotectants were present in the media, whereas without cryoprotectants oocytes retained their developmental capacity. We conclude that cryoprotectants may extract some toxic substances from the aluminium tubes. Future studies should evaluate another inert tube material to be used for self-pressurized rapid freezing.

GRABENBAUER M, HONG-MEI H, HUEBINGER J (2014): Cryofixation by Self-Pressurized Rapid Freezing. *Methods Mol Biol* **1117**, 173 - 191.

MIKOLAJEWSKA N, MÜLLER K, NIŻAŃSKI WI, JEWGENOW K (2012): Vitrification of Domestic Cat Oocytes – Effect on Viability and Integrity of Subcellular Structures. *Reprod Domest Anim* **47** Suppl 6, 295 - 299.

## **Genetic analysis of coagulation Factor VII and its correlation with Elephant Endotheliotropic Herpesvirus in Asian elephants (*Elephas maximus*)**

FONTES SÓNIA J, SCHMIDT ANKE, FICKEL JÖRNS, HILDEBRANDT THOMAS B

Leibniz Institute for Zoo and Wildlife Research (IZW), Dept. of Reproduction Management, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; fontes@izw-berlin.de

Elephant endotheliotropic herpes virus (EEHV) is responsible for the death of up to 50 % of all captive born Asian elephants. Common pathological findings in all necropsy cases include an acute haemorrhagic syndrome, with the presence of pericardial effusion and haemorrhages in histological sections of several organs. Preliminary data shows that the majority of these cases are clustered in groups of related captive animals. Recently, one captive Asian elephant bull living in Australia was accidentally diagnosed with coagulation Factor VII (FVII) deficiency, with a single homozygous point mutation. Three of his offspring were also reported to be heterozygous for this mutation.

To better understand if a hereditary coagulation disorder is involved in the onset or the fatal outcome of this haemorrhagic disease in the European population of Asian elephants, we measured the coagulation time of 16 individuals. As results presented reasonable variability among the tested population, we decided to further investigate. Therefore, we analysed 37 animals from 15 Zoological institutions; frozen tissue (liver and myocardium) or blood (EDTA tubes) samples were studied for coagulation FVII gene mutations in these individuals, which included four EEHV haemorrhagic disease fatal cases. Out of the eight exons of the FVII gene, we successfully amplified and sequenced seven, and found several single nucleotide polymorphism (SNP) in four exons among the individuals investigated.

We hypothesize that Factor VII is correlated with the outcome of the EEHV infection and responsible for the fatal haemorrhagic disease. All fatal cases reported in Europe should show a genetic pattern different from the rest of the Asian elephant population. Although establishing a causative relation between Factor VII mutation and EEHV-HD is not yet possible, we here present the preliminary results found during this investigation.

This project is part of a doctoral study supported and co-founded by the European Association of Zoo and Aquaria's (EAZA) Elephant Taxon Advisory Group (TAG).

## Genetic background influences dominance ranks in male baboons

FRANZ MATHIAS<sup>1</sup>, ONYANGO PATRICK<sup>2</sup>, TUNG JENNY<sup>3</sup>, ALBERTS SUSAN C<sup>4</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Dept. of Wildlife Diseases, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; m.franz@izw-berlin.de

<sup>2</sup>Maseno University, Department of Zoology, P.O. Box 3275-40100, Kisumu, KENYA

<sup>3</sup>Duke University, Department of Evolutionary Anthropology, 125 Science Drive, Durham, NC 27708, USA

<sup>4</sup>Duke University, Department of Biology, 125 Science Drive, Durham, NC 27708, USA

A common consequence of linear dominance hierarchies in social animals are benefits for high ranking individuals, e.g. in form of increased access to limited ecological resources or mating opportunities. Yet, our understanding of what determines the attainment of high dominance ranks is still limited. In many cases there are clear indications that ontogenetic development of weaponry and body condition influences the rank of individuals. However, whether genetic background can also influence dominance ranks is largely unexplored. Assessing the impact of genetic background in wild animals is challenging due to the need of controlling for other sources of variation such as age-related changes in dominance ranks. To address this challenge we developed a novel hierarchical modelling approach that operates within a Bayesian model fitting framework. We applied this approach to a long-term data set of wild baboons collected between 1996 and 2011 by the Amboseli Baboon Research Project. Our study population is particularly well suited for investigating potential effects of genetic background on dominance ranks. Variance in genetic background in Amboseli arises from the natural admixture between yellow baboons (*Papio cynocephalus*) and anubis baboons (*P. anubis*), which interbreed freely and produce viable and fertile offspring. Previous work on our population showed that, after controlling for dominance rank, males with more anubis admixture have higher mating success. However, it is still unclear whether more anubis-like males are also more successful in attaining high dominance ranks – or on the contrary whether these males are less successful in attaining high ranks and therefore compensate this lower success in high rank attainment by increased mating activity. The application of our hierarchical model shows that, after controlling for age-related changes, more anubis-like males attain higher dominance ranks than more yellow-like males. This finding supports the idea that genetic background influences the attainment of dominance ranks in wild male baboons. Furthermore, our results add to existing information of genetic influences on a whole suite of life history and behavioural traits including maturation age, mating success and winner and loser effects in male baboons. Thus, our findings refine our understanding of evolutionary changes that are occurring at the hybrid zone between wild populations of yellow and anubis baboons.

## **Digestibility in greylag geese (*Anser anser*): the role of social context**

FRIGERIO DIDONE<sup>1,2</sup>, PUEHRINGER-STURMAYR VERENA<sup>1,2</sup>, HEMETSBERGER JOSEF<sup>1,2</sup>, IAIZA LARA<sup>3</sup>, MASON FEDERICO<sup>3</sup>, SARNATARO CHIARA<sup>3</sup>, FABRO CARLA<sup>3</sup>, FILACORDA STEFANO<sup>3</sup>

<sup>1</sup>Core facility KLF for Behaviour and Cognition, University of Vienna, Fischerau 11, 4645 Grünau im Almtal, AUSTRIA; didone.frigerio@univie.ac.at

<sup>2</sup>Department of Behavioural Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, AUSTRIA

<sup>3</sup>Department for Agro-Food, Environmental and Animal Science, University of Udine, Via delle Scienze 206, 3310 Udine, ITALY

Social context is known to modulate physiology, behaviour, fertility and immune system of group living animals. Furthermore, individuals' nutritional strategies may affect their performance and impact their social interactions. In the present study we discuss the results of a pilot study conducted on N = 38 individuals of the semi-tame, long-term monitored and individually marked flock of Greylag geese (*Anser anser*) at the Konrad Lorenz Research Station in Upper Austria. Focal birds belonged to different social categories within the flock, i.e. paired with and without offspring, single and juveniles (five males and three to five females per category). The animals are well habituated to the close presence of humans and are used to get fed twice a day on the meadow in front of the Research Station. During 9 consecutive days in winter 2017, when the snow pack was closed, after standardization of the provided food, a total number of 184 individual droppings were collected from the focal individuals (mean = 4,8; SD = 0,4). Samples were analysed by determining the apparent digestibility of total organic matter. Preliminary results show similarities between males and females but hint at differences depending on the social category with parental individuals showing higher digestive capability than unpaired individuals or paired without offspring. We discuss our findings with respect to the complex relationships between social status, physiology and the capacity of digesting food, extracting nutrients and improving fitness.

## **Assessment and conservation of genetic diversity in captive and wild populations of the Golden-headed lion tamarin, using pedigree and molecular data**

GALBUSERA PETER<sup>1</sup>, LEUS KRISTIN<sup>1</sup>, DE VLEESCHOUWER KRISTEL<sup>1</sup>, HELSEN PHILIPPE<sup>1</sup>, VERMEULEN ELKE<sup>1</sup>, MORAES ANDREIA<sup>2</sup>, GRATIVOL ADRIANA<sup>2</sup>, RUIZ-MIRANDA CARLOS<sup>2</sup>, RABOY BECKY<sup>3</sup>, OLIVEIRA LEONARDO<sup>4</sup>, DIETZ JAMES<sup>5</sup>

<sup>1</sup>Centre for Research and Conservation, Royal Zoological Society of Antwerp, Koningin Astridplein 20-26, 2018 Antwerp, BELGIUM; peter.galbusera@kmda.org

<sup>2</sup>Laboratório de Ciências Ambientais, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego 2000, CEP 28013-602, Campos do Goytacazes, RJ, BRAZIL

<sup>3</sup>Department of Ecology and Evolutionary Biology, University of Toronto, 25 Harbord Street, RW 508, Toronto M5S 3G5, ON, CANADA

<sup>4</sup>Pós-graduação em Ecologia e Conservação da Biodiversidade, Universidade Estadual de Santa Cruz, UESC, Salobrinho, CEP 45662-900, Ilhéus, BA, BRAZIL

<sup>5</sup>Program in Behavior, Ecology, Evolution and Systematics, University of Maryland, College Park, MD 20742, USA

The Golden-headed lion tamarin (Ghlt) is an endangered primate species, with a mandate from the Brazilian government for a genetically and demographically self-sustainable captive population with the primary role of assurance. The secondary roles are conservation education, research and fundraising. The genetic status of captive populations is typically based on the pedigree and founder assumptions. Assuming Ghlt founders are unrelated, the world captive population is large enough (about 520 individuals) to ensure conservation of 90 % of gene diversity (GD) of the wild population for 100 years. It's current GD is 98 % in the world population and 96 % in the European (EEP) population. However, when taking into account founder relatedness (as estimated by molecular markers), this EEP estimate is reduced to about 86 %. Using the same genetic markers (microsatellite DNA), we also assessed GD in wild populations. Overall, the results suggest that these populations have a relatively low GD and high population genetic structure, raising questions about actual functional landscape connectivity. Assignment of captive animals to the wild subpopulations, suggests that captive animals may have originated from cross-breeding, resulting in relatively high GD compared to the wild. The continued loss of habitat makes it desirable to continue managing the self-sustainable world captive population as an insurance population. Future genetic research will further optimize this management in captive as well as wild populations.

## Being born late in the active season: implications for growth, fattening, torpor use and fertility

GIROUD SYLVAIN, GERRITSMANN HANNO, STALDER GABRIELLE, BLANC STÉPHANE\*, RUF THOMAS\*

University of Veterinary Medicine Vienna, Research Institute of Wildlife Ecology, Department of Integrative Biology and Evolution, Savoyenstraße 1, 1160 Vienna, AUSTRIA; sylvain.giroud@vetmeduni.ac.at

\*These authors contributed equally to the work

Prior to winter, juvenile hibernators have to allocate energy to both growth and fattening, to survive winter hibernation and to avoid possible disadvantages during the first reproductive season. An adverse early environment can impose severe constraints on development resulting in stunted, poorly performing individuals, namely the ‘silver-spoon effect’ (GRAFEN, 1988; MONAGHAN, 2008). Being born late in the active season may have important effects on growth and fattening. Torpor is thought to sustain these processes in young individuals (GEISER 2008; GIROUD *et al.*, 2014). We tested the hypothesis that late-born (‘LB’) female juvenile garden dormice (*Eliomys quercinus*) (i) grow and fatten at higher rates, (ii) reach similar pre-hibernation levels of body size and fat reserves due to (iii) an increased use of torpor, (iv) show similar hibernating patterns and (v) reproduce less than early born (‘EB’) individuals. We found that, during the pre-weaning phase, young from early and late litters mainly grew structurally and gained body mass at similar rates. However, at early post-weaning phase, LB juveniles were smaller but fatter, then grew and gained mass twice as fast as EB individuals during their post-weaning period. Accelerated growth was sustained by an increased food intake and reduced activity duration in LB juveniles, leading to body sizes similar to EB individuals prior to hibernation. Torpor use was low during intensive growth (*i.e.* the first weeks of body mass gain), but increased after the termination of growth, during pre-hibernation fattening. LB juveniles increased their torpor use to a greater extent and showed 10 %-lower pre-hibernation fat reserves, compared to EB individuals. Intermittent fasting (IF) had no effect on fat stores prior to hibernation, neither in EB nor in LB juveniles. Apart from a four-week difference in their hibernation duration, EB and LB juveniles showed similar hibernating patterns. Surprisingly, after their first winter, LB dormice reproduced overall more than EB individuals, and dormice that were IF showed a lower fertility than individuals that were fed *ad-libitum*. Hence, our results suggest that torpor is incompatible with structural growth, but instead promotes pre-hibernation fattening late in the season. This study further supports, rather than a silver spoon effect, the ‘internal predictive adaptive response’ hypothesis, which predicts that individuals born in poor conditions, *i.e.* late in the season and/or with low food availability, should start to reproduce earlier if they are likely to have reduced performance later in life (NETTLE *et al.*, 2013).

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## **Reproductive effort in giraffe females – does it change with increasing experience?**

GLONEKOVÁ MARKÉTA<sup>1</sup>, BRANDLOVÁ KAROLÍNA<sup>1,4</sup>, PLUHÁČEK JAN<sup>2,3</sup>

<sup>1</sup>Czech University of Life Sciences Prague, Department of Animal Science and Food Processing in Tropics and Subtropics, Kamýcká 129, 165 21, Praha 6, CZECH REPUBLIC, glonekovamarketa@gmail.com

<sup>2</sup>Institute of Animal Science, Department of Ethology, Přátelství 815, 104 00 Praha-Uhřetěves, CZECH REPUBLIC

<sup>3</sup>Ostrava Zoo, Michálkovická 194, 710 00 Ostrava, CZECH REPUBLIC

<sup>4</sup>Dvůr Králové Zoo, Štefánikova 1029, 544 01 Dvůr Králové nad Labem, CZECH REPUBLIC

The reproductive effort of mammalian females could change during their ontogeny. The difference may be related to the age and experience (parity). Four hypotheses have been postulated to explain changes in reproductive effort of the females. The residual reproductive value hypothesis and the terminal investment hypothesis suppose higher reproductive effort in older and more experienced females, respectively. Targeted reproductive effort hypothesis predicts lower reproductive effort in more experienced females and the senescence hypothesis predicts lower reproductive effort in older females. We have investigated acceptance rate, the probability of successful nursing bout, the time devoted to nursing and nursing frequency as the predictor of mothers' reproductive effort to test these hypotheses in captive giraffes (25 females, 52 calves in four zoos in the Czech Republic).

The acceptance rate, probability of successful nursing bout, the nursing bout frequency as well as the total time devoted to nursing increased with an increasing parity of the female which supported the residual reproductive value hypothesis or the terminal investment hypothesis. These hypotheses expect to increase the reproductive effort toward the end of her life-span, when the number of the offspring expected to rear in the future is low. Therefore, the female invests more to the current young as was found in this study.

## Effects of social density during the rut and arousal at mother-offspring separation on male and female vocalisation traits in Siberian wapiti

GOLOSOVA OLGA S<sup>1</sup>, SIBIRYAKOVA OLGA V<sup>1</sup>, VOLODIN ILYA A<sup>1,2</sup>, VOLODINA ELENA V<sup>2</sup>

<sup>1</sup>Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Vorobievsky Gory, 1/12, Moscow, 119234, RUSSIA, golosova95@yandex.ru

<sup>2</sup>Scientific Research Department, Moscow Zoo, B. Gruzinskaya str., 1, Moscow, 123242, RUSSIA

Vocalisations of mammals represent potential indicators of animal welfare. Although the Siberian wapiti *Cervus elaphus sibiricus* is the most important cervid species among farmed animals of Russia, China and Kazakhstan, welfare standards are not yet established for this mammal. Local density of animals during the rut may affect vocal activity and the acoustics of rutting vocalizations in males. At the same time, separation of offspring from their mothers may affect the acoustic characteristics of females. We compared the acoustics of male rutting calls for two farmed populations that differed in density (0.08 deer/ha vs. 2.0 deer/ha), 103 and 123 calls per population. Individuals could not be identified, because calls were collected using automated recording systems (SongMeter SM2+) in September-October of 2013 and 2015, respectively for the low and high density population. To avoid the influence of pattern of fundamental frequency modulation on the acoustics, we took for acoustic analyses only calls with a “trapeze” pattern of modulation. In the high density population, calls were shorter (2.94 s vs. 3.15 s,  $p < 0.01$ ), higher in the mean fundamental frequency (1.20 kHz vs. 1.12 kHz,  $p < 0.001$ ) and higher in the maximum fundamental frequency (1.46 kHz vs. 1.34 kHz,  $p < 0.001$ ) than in the low density population. The peak frequency (1.45 kHz vs. 1.49 kHz) did not differ. We also compared mean individual values of acoustic variables for female calls emitted towards milk-dependent offspring (< 1 month) and milk-independent offspring (5-6 months) during mother-offspring separations, recorded respectively in June and in December 2015 in the high density population. In females with milk-dependent offspring (21 mothers, 1-15 calls per mother, 133 calls in total) calls were shorter (0.40 s vs. 0.56 s,  $p < 0.01$ ), lower in the peak frequency (1.78 kHz vs. 2.20 kHz,  $p < 0.05$ ), higher in the mean fundamental frequency (1.25 kHz vs. 0.99 kHz,  $p < 0.05$ ) and marginally higher in the maximum fundamental frequency (1.63 kHz vs. 1.44 kHz,  $p = 0.06$ ) than in females with milk-independent offspring (9 mothers, 14-15 calls per mother, 134 calls in total). We suggest that in Siberian wapiti, elevated emotional arousal (in males from low to high social density during the rut and in females from milk-independent to milk-dependent offspring) results in call shortening and elevation of fundamental frequencies. This will be useful to estimate discomfort and improve animal welfare on deer farms.

Support: Russian Science Foundation, grant No 14-14-00237.

## **Vocal variability in chicks of three crane species: indicators of age, individuality, sex, hormone value, hunger and cooling**

GONCHAROVA MARIA<sup>1</sup>, KLENOVA ANNA<sup>2</sup>

<sup>1</sup>National Research Center for Hematology, Novy Zykovsky proezd 4a, 125167, Moscow, RUSSIA; goncharova.maria.v@gmail.com

<sup>2</sup>Lomonosov Moscow State University, Faculty of Biology, Department of Vertebrate Zoology, Leninskie gory 1-12, 119234, Moscow, RUSSIA

It is well known that vocal signals play a key role in parent-offspring interaction. Therefore it is very interesting to figure out how chicks convey information about their individuality and condition, and how their vocal behaviour changes during development. Cranes are interesting subjects for such research, because they rear one chick per season, have prolonged parental care and as most of them are rare in nature, the knowledge about development of crane chicks is important for reintroduction programmes. Here we studied three species: the Demoiselle (*Anthropoides virgo*, DC), Red-crowned (*Grus japonensis*, RC) and Siberian (*G. leucogeranus*, SC) cranes, that differ in their growth and development rates, vocal characteristics and a few other behavioural traits. We regularly recorded calls, conducted experiments with food and warmth deprivation, collected blood samples and measured the testosterone level from hatching up to first year of life. We found that chicks of all three species retain juvenile temporal-frequency characteristics of calls during first year of life and gain the adult vocal repertoire through the stage of voice-breaking when they produce both adult low-frequency and chick high-frequency vocalisations. The onset of voice breaking occurred at  $2.3 \pm 1.5$  months in DC ( $n = 14$ ), at  $7 \pm 2$  months in RC ( $n = 24$ ) and at  $8.1 \pm 1.6$  months in SC ( $n = 10$ ) and was slightly related with the end of active morphological growth. The end of voice breaking in all species occurred at the same time (at 9-12 months); that coincides with a dramatic increase of plasma testosterone level and breakup of parent-chick bond in nature. We also found that vocal indicators of sex were poorly expressed during first six months of crane's life i.e. vocal sexual dimorphism reported for adult cranes develops later, maybe during sex maturation. However, expression of individual features considerably and significantly increased with fledging and transition to migratory lifestyle ( $n = 11$  for each species, DFA); that can contribute to increasing of parent-chick recognition success in dense flocks. We also found that almost all frequency variables in hungry and cooled SC chicks were significantly higher than in control ones ( $n = 9$  food deprived and  $n = 10$  warmth deprived SC chicks; e.g. maximum fundamental frequency in hunger chicks:  $3.41 \pm 0.53$  kHz, in satiated ones:  $3.34 \pm 0.53$  kHz). Calls of hunger and cooled chicks were also longer, noisier and followed each other through smaller gaps. We discuss how the vocal variability we found is related with ecological traits of different crane species.

Supported by the Russian Science Foundation (grant 14-14-00237).

## Urban structures affect the spread of a wildlife disease

GRAS PIERRE<sup>1,2</sup>, KNUTH SARAH<sup>1,3</sup>, BÖRNER KONSTANTIN<sup>1</sup>, MARESCOT LUCILE<sup>1</sup>, BENHAIEM SARAH<sup>1</sup>, AUE ANGELIKA<sup>4</sup>, WITTSTATT ULRICH<sup>4</sup>, KLEINSCHMIDT BIRGIT<sup>3</sup>, KRAMER-SCHADT STEPHANIE<sup>1,2</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Dept. of Evolutionary Ecology, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; gras@izw-berlin.de

<sup>2</sup>Berlin-Brandenburg Institute of Advanced Biodiversity Research, Altensteinstraße 34, 14195 Berlin, GERMANY

<sup>3</sup>Technische Universität Berlin, Geoinformation in Environmental Planning Lab, Straße des 17. Juni 145, 10623 Berlin, GERMANY

<sup>4</sup>Landeslabor Berlin-Brandenburg, Infektionsdiagnostik, Invalidenstr. 60, 10557 Berlin, GERMANY

Urbanization is increasing worldwide, thereby also enlarging the human-wildlife interface with positive as well as negative aspects for both humans and wildlife. One negative aspect is the spread of diseases that may spill over from pets to wildlife and vice-versa. As urban structures determine the distribution and movement of wild animals, they should have a key influence on disease spread. However, very little is known in general about the role of urban structures on disease spread. To guide management, assessing the role of urban structures in hampering or promoting the spread of diseases is thus of paramount importance.

Canine distemper is an important, virulent wildlife disease, causing high mortality in its hosts, particularly in juveniles. Airborne transmission and high virulence of canine distemper viruses (CDV) cause rapid and wave-like epidemics and complicating effective disease management. Moreover, effects of urban structures on CDV spread as well as potential physical barriers hindering spread are largely unknown.

Between 2007 and 2013, two epidemic waves of canine distemper hit the urban fox (*Vulpes vulpes*) population of Berlin, Germany. We extracted information about CDV seroprevalence, individual sex and age for 778 urban red fox carcasses collected by the state laboratory Berlin-Brandenburg during these seven years. We geo-located the carcass positions and extracted landscape variables at different spatial scales (areal of 30 ha, 80 ha, 300 ha) to assess the impact of urban structure diversity (e.g. landscape type richness) and the shares of green and grey infrastructures within each buffer. We used generalized linear mixed models (GLMM) with a binary response variable (seropositive vs. seronegative sample) and our environmental predictors as well as sex and age as explanatory variables. Year and district were entered as random effect.

Our results indicate that predictors derived at the 80 ha scale were most informative for describing landscape structures affecting the spread of CDV seroprevalence in foxes. CDV seroprevalence decreased with the increasing share of grey infrastructure, hence urban, anthropogenic structures might hamper CDV spread. Finally, we mapped the risk of CDV prevalence in wildlife across the city and identified areas harbouring predominantly seropositive or seronegative foxes as guidance for wildlife management.

## **Advantages of scat detection dogs: A case study from related mustelid species with identical diet**

GRIMM-SEYFARTH ANNEGRET<sup>1,2</sup>, ZARZYCKA ALEKSANDRA<sup>1</sup>, KLENKE REINHARD<sup>1</sup>

<sup>1</sup>UFZ – Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstr. 15, 04318 Leipzig, GERMANY; annegret.grimm@ufz.de

<sup>2</sup>Wildlife Detection Dogs e.V., Sofienstraße 5, 74867 Neunkirchen, GERMANY

Many animal and plant species of interest in nature conservation and human-wildlife-interactions are hard to find and therefore difficult to monitor. This is especially true for elusive or nocturnal species. Since many elusive species are conflict-laden but threatened or endangered at the same time, it is even more challenging to analyse these species' distributions or life-history which would be important for their conservation and conflict management. One of those species is the Eurasian otter (*Lutra lutra*). Since its diet mainly consists of fish, the Eurasian otter is considered as one of the main competitors to pond farmers. Despite scat monitoring has been successfully performed throughout Europe in the past, it recently became very difficult with the invasive mink (*Neovison vison*) spreading through the same semi-aquatic habitats in Europe. As scats of otters, mink and possibly other syntopic carnivores resemble in shape and colour, visual searches highly likely overestimate otter presence. Therefore, we trained four scat detection dogs to verify the target species in the field. It has been already shown that dogs could distinguish between sympatric, closely related species and even single individuals. However, we now examined whether scat detection dogs are also able to discriminate between related mustelid species even when they have fed on the same diet. We also compared detection and discrimination abilities of scat detection dogs and humans.

In a first lab test, we examined discrimination abilities of dogs and humans. Dogs correctly alerted at 100 % of the samples of their target species and correctly ignored 90 % (80-100 % depending on the dog) of the non-target species. In comparison, humans correctly assigned 80 % (55-100 % depending on the person) of the scat samples to the species. Human assignment was highly biased by scat colour which is not an indicator of the species. Moreover, their correct assignment increased with both increasing level of experience and when they have been trained by a dog-human-team in the field instead of a human alone. We then tested detection dogs and humans in the field. We recorded that dogs found more samples of the target species than humans; they searched a longer transect in the same time and did not differentiate between fresh and old scats whereas humans frequently sampled non-target species and old (bright) scats. Therefore, we conclude that the use of scat detection dogs can increase both the efficiency and the data quality of species monitoring by means of scats.

## Scat detection dog for Balkan lynx (*Lynx lynx balcanicus* syn. *martinoi*) conservation

HARTL CHRISTOPH<sup>1</sup>, HOFMANN TIM<sup>1</sup>, IVANOV GJORGJE<sup>2</sup>, MELOVSKI DIME<sup>2</sup>

<sup>1</sup>SniffX, Bernauer Straße 140, 83209 Prien am Chiemsee, GERMANY; mail@sniffx.de

<sup>2</sup>Macedonian Ecological Society, Boris Trajkovski 7/9a, Skopje 1000, MACEDONIA (EJRM); ivanov@mes.org.mk; melovskid@mes.org.mk

Balkan lynx (*Lynx lynx balcanicus* syn. *martinoi*) population is in severe decline. Research is challenging since the individuals have huge territories and display an elusive behaviour. As a consequence, there is still a lack of knowledge regarding the Ecology and the requirements for a successful conservation.

One of the most important regions for the reproduction of the subspecies is Mavrovo National Park in the Republic of Macedonia. Until now, these lynx are mainly monitored with camera traps that are most often set on forest roads, hiking or game trails. Moreover, live-traps are used to capture individuals for telemetry studies. Up to now, only five lynx were caught in seven years. Hence, a successful conservation demands more information about the population especially at the genetic level. Detection dogs have been proofed to be very efficient in finding high-quality carnivore faeces suitable for DNA-analysis. We tested whether the method was applicable in Balkan lynx conservation biology and trained a dog (Magyar Vizsla breed) to detect lynx scat. Within the 42 km<sup>2</sup> sized study area near Izvor (Kicevo), we randomly placed 42 transects of one kilometre length each. 21 of them were designed to follow paths and forest roads; the other half was distributed randomly in the forest.

The detection dog covered a total distance of 87.5 km, 56.8 km on transects in the field and 30.7 km on forest road transects. We collected four scat samples of which two were confirmed by DNA-analysis, the other two were of insufficient quality. One sample was of the mitochondrial haplotype four which was previously only found in the Carpathian lynx population and spread into the Alps by introduced individuals. Besides, the dogs detected three lynx kill sites and we were able to collect three additional proven lynx scat samples there, two of them also displaying haplotype four. At one site, the first female Macedonian lynx was caught and radio-collared subsequently.

In conclusion, detection dog work is a very promising method to gather more information about the Balkan lynx and should be included in future research projects.

## Soaring again in France: genetic monitoring to evaluate the reintroduction process of Eurasian Black Vultures

HELSEN PHILIPPE<sup>1</sup>, HUYGHE MARLEEN<sup>1</sup>, GALBUSERA PETER<sup>1</sup> NEOUZE RAPHAEL<sup>3</sup>, TESSIER CHRISTIAN<sup>5</sup>, HENRIQUET SYLVAIN<sup>2</sup>, DURIEZ OLIVIER<sup>3,4</sup>, SARRAZIN FRANCOIS<sup>6</sup>

<sup>1</sup>Centre for Research and Conservation, Royal Zoological Society of Antwerp, Koningin Astridplein 20-26, 2000 Antwerp, BELGIUM; philippe.helsen@kmda.org

<sup>2</sup>LPO Vautours du Verdon, Boulevard Saint Michel 5, 04120 Castellane, FRANCE

<sup>3</sup>LPO Grands Causses, Le bourg, 12720 Peyreleau, FRANCE

<sup>4</sup>Centre d'Ecologie Fonctionnelle & Evolution, Route de Mende 1919, 34293 Montpellier, FRANCE

<sup>5</sup>Association Vautours en Baronnie, Mairie, 26510 Rémuzat, FRANCE

<sup>6</sup>MNHN, Rue Cuvier 57, 75005 Paris, FRANCE

Huge losses in biodiversity became a key feature to describe what happened during the last two centuries. At present, many species are indeed at the brink of extinction, which led to a rise in conservation initiatives. Reintroductions have gained importance in many of such initiatives. Not all restorations however seemed to be as efficient as originally planned. Largely driven by vast investments on the field, vulture reintroduction programs can be regarded showcases on what is needed to make a conservation initiative into a success. Here we describe how “basic” genetic research increases our comprehension on factors contributing to the success of reintroduction projects with a special focus on the restoration of the Eurasian black vulture (*Aegypius monachus*) in France led by LPO and Vautour en Baronnie.

With the recent disappearance of “Central European” vulture colonies, both a critical actor within the central European ecosystem was lost and a new barrier for gene flow was introduced. Accordingly, the Iberian population became isolated from more eastern populations. Aiming to restore both, ecosystem services and gene flow, Eurasian black vultures originating both from Spanish rehabilitation centers and ex-situ breeding facilities, have been released at three different locations in France (n = 127). Post-release monitoring as well as screening of wild born individuals (first breeding event in 1996 and > 200 wild hatches at present) allows testing which birds (or individual characteristics of these birds) contributed most to the success of the project. Here feather samples of over 250 birds were used to study genetic relatedness as defined by twenty microsatellite markers. Whereas sibling relatedness gave extra insights on the species' biology, parentage analysis revealed that both resource populations (i.e. rehabilitation and ex-situ captive bred) contributed equally during the first stages of the project, more precisely when setting up new colonies. In concordance with what is known from juvenile dispersal in raptors, reintroduced males produced more (> 3x) local offspring than their female counterparts. Another important parameter within reintroduction projects is the relative reproductive success of wild hatched over reintroduced birds. With individual identifications being challenging for this species, it is impossible to evaluate this directly. At present reintroductions are in their final stage. As such we evaluated trends in this relative reproductive success to advice on optimal timing to downscale reintroduction efforts in future reintroduction projects, which have already been planned for this species and might be needed for many other vulture species in the near future.

## **Of (s)cats and dogs: Detection dogs allow for systematic non-invasive collection of DNA samples of Eurasian Lynx (*Lynx lynx*)**

HOLLERBACH LAURA<sup>1</sup>, HEURICH MARCO<sup>2,3</sup>, GAHBAUER MARTIN<sup>2</sup>, JEB ELENA<sup>4</sup>, REINERS TOBIAS ERIK<sup>1</sup>, COCCHIARARO BERARDINO<sup>1</sup>, JÜNGLING HANNAH<sup>1</sup>, TIESMEYER ANNIKA<sup>1</sup>, JOKISCH SUSANNE<sup>5</sup>, NOWAK CARSTEN<sup>1</sup>

<sup>1</sup>Senckenberg Research Institute and Natural History Museum, Conservation Genetics Group, Clamecystraße 12, 63571 Gelnhausen, GERMANY; laura.hollerbach@senckenberg.de

<sup>2</sup>Bavarian Forest National Park, Department of Research and Documentation, Freyunger Straße 2, 94481 Grafenau, GERMANY

<sup>3</sup>University of Freiburg, Chair of Wildlife Ecology and Management, Faculty of Environment and Natural Resources, Tennenbacher Straße 4, 79106 Freiburg, GERMANY

<sup>4</sup>Von-Escherte-Straße 2, 30539 Hannover, GERMANY

<sup>5</sup>Hessian Agency for Nature Conservation, Environment and Geology, Department of Conservation, Europastraße 10, 35394 Gießen, GERMANY

Genetic analyses are a valuable tool to answer a range of conservation and management relevant questions. DNA samples of lynx in their natural habitat are difficult to obtain because lynx are rare and occupy large home ranges. Besides, scats and hairs cannot be reliably identified in the field. There have been approaches to collect hair samples through hair traps. However, the effort is high for this method and success rates are low. Consequently, the development of alternative methods for DNA sample collection of lynx is of great interest.

The use of trained dogs in order to obtain information about one or more target species has gained increasing attention among researchers and managers during the last years. Dogs have been successfully used to detect scats of species such as bobcat (*Lynx rufus*) or Canada lynx (*Lynx canadensis*) as a basis for genetic analyses.

Here, we aim to compare the success rate of scat detection dogs with hair trapping. For four weeks, we worked with two specially trained detection dogs to find scat samples of lynx in the Bavarian Forest National Park, Germany. We set up 44 adjacent 2 x 2 km grids cells across the national park. Each dog team surveyed 22 transects and was required to survey in each grid cell. Transects were mostly circular and largely consisted of forest roads and trails. Scats that the dogs indicated were collected and genetically analysed. In parallel to the dog survey, we set up one hair trap treated with valerian tincture in every grid cell. Hair traps were inspected weekly, for a total of four times.

The dogs detected 50 scats and two hair samples that were confirmed as lynx by mitochondrial DNA sequencing. Genotyping of samples up to the individual level was successful for 28 of these samples and identified 11 individuals of which four were male and six were female. The sex of one individual could not be determined. First results indicate that detection dogs collected nearly six times more confirmed DNA samples of lynx than hair traps.

Our results show that trained detection dogs have great potential to increase the number of non-invasive DNA samples of lynx and therefore improve data availability for lynx monitoring and research.

## Assessing the use of iDNA from haematophagous leeches as an alternative tool for wildlife monitoring

HÖRIG LISA<sup>1,2</sup>, ALEX CRAMPTON-PLATT<sup>2</sup>, AXTNER JAN<sup>2</sup>, BIN MOHAMED AZLAN<sup>2</sup>, WILTING ANDREAS<sup>2</sup>

<sup>1</sup>University of Potsdam, Institute for Biochemistry & Biology Maulbeeralle 1, 14469 Potsdam, GERMANY; hoerig@uni-potsdam.de

<sup>2</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Junior Research Group: Biodiversity and Biogeography of Southeast Asia, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY

Globally biodiversity is declining at an alarming rate and thus the conservation of vulnerable species and their habitats is of crucial importance. The development of effective management plans requires long-term monitoring of the threatened populations. But especially in tropical rainforests studying the often secretive species in remote areas poses a challenge for biologists and conservationists. In recent years, invertebrate-derived DNA (iDNA) in combination with high throughput sequencing techniques has been proposed as a cost efficient and powerful tool for the monitoring of vertebrate species. In iDNA studies vertebrate genetic material is extracted from mostly blood-sucking invertebrates or carrion flies and obtained sequences are identified using a metabarcoding framework to receive information about their hosts. Here we sequenced a total of 1532 haematophagous leeches in 246 samples from the Deramakot Forest Reserve, a logging concession in Sabah, Malaysia. Leeches were collected in close vicinity (10 - 20m) around 64 camera-traps. Species identification was realized through PCR amplification of three mitochondrial markers (*12s*, *16S* & *Cyt-b*) using generic vertebrate primers, followed by sequencing on an Illumina Miseq. We applied double barcoding to identify tag jumps and false positives. About 50 % of the leech samples were host positive. Overall we identified 22 mammal species, two amphibians and one bird. In contrast to the parallel on-going camera-trapping our approach recorded the threatened Binturong *Arctitis binturong* and the two frog species *Kalophrynus barioensis* and *Kalophrynus pleurostigma*. We were further able to distinguish between two species of mouse deer (*Tragulus javanicus* and *T. napu*), which cannot reliably be discriminated on camera-trap photos. Thus, our results are a promising indication that leech-derived iDNA may overcome difficulties in surveying and thus monitoring secretive species in tropical rainforests. Even with a comparatively small sample (116 host-positive leech samples compared to 3177 independent camera-trap detections) we identified more than 50 % of the camera trapped species and even detected additional ones. However, our results also show that collection of a much greater number of leeches is necessary for a comprehensive species assessment. If such intensive collection is not feasible we still see a great value in complementing camera-trapping studies with iDNA analysis, as certain taxa can be better recorded or identified.

## **Monitoring of luteal activity in dromedary camels (*Camelus dromedarius*) by using non-invasive quantification of faecal progestagens metabolites after GnRH stimulation**

ISMAIL OMAR (SID AHMED)<sup>1</sup>, SKIDMORE JULIAN<sup>2</sup>, MÖSTL ERICH<sup>3</sup>

<sup>1</sup>AniCura Jeløy Dyreklinikk, Nesveien 440, 1514 Moss, NORWAY; omarvet78@gmail.com

<sup>2</sup>Camel Reproduction Centre, P.O. Box 79914, Dubai, UAE

<sup>3</sup>Department of Biomedical Sciences, Institute of Medical, Biochemistry, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, AUSTRIA

Within the last two decades there has been a rapid advancement in EIA-techniques to monitor gonadal and adrenal steroids non-invasively in both domestic and wildlife animals. However, the only used method for profiling of reproductive hormones in dromedary including progesterone (P4) is invasive (serum). On the other hand, non-invasive measurement of different adrenal and gonadal steroid metabolites are widely now applied as most popular tool for the assessment of the animal welfare and reproductive status. However, due to species variations in the excreted amount and the chemical configurations in steroid metabolites nucleus in faeces, a validation test constitute an absolute necessity to check the suitability of the EIA in certain animal species under investigation. Therefore, we aimed to validate two EIAs having 4-pregnen-3,20-dione (5a) and 5 $\beta$ -pregnan-3 $\alpha$ ,20 $\alpha$ -diol (5b) configurations for the non-invasive monitoring of faecal progestagen metabolites (FPM) following GnRH injection in dromedary.

Four non-pregnant dromedaries (age: 5-12 years) were selected from the camel reproduction centre in Dubai, UAE. The animals were injected intramuscularly with 2 ml GnRH (Gonavet Veyx - Pharma GmbH, Germany). Frequent blood samples were collected via permanent catheter in plain Vacutainer at day 0 (GnRH injection) and post injection for 15 consecutive days. Faecal samples were collected after spontaneous defecation for 18 consecutive days (post GnRH injection). Both serum and faeces were stored at -20 °C, extracted, and analysed with a respective EIA. Individual variations were observed in both serum and faecal metabolites concerning authentic P4 and FPM. Baseline serum P4 levels were 0.01 to 0.11 (Mean  $\pm$  SEM: 0.05  $\pm$  0.03) ng/ml. Serum peak concentrations were 0.75 to 1.65 (Mean  $\pm$  SEM: 1.17  $\pm$  0.19) ng/ml. Peak concentrations were reached 10.3 days after GnRH injection and returned to baseline levels within 11 to 15 days. Baseline concentrations of FPM ranged from 39.66 to 69.41 (Mean  $\pm$  SEM: 50.47  $\pm$  5.51) and from 192.7 to 1108.690 (Mean  $\pm$  SEM: 546.94  $\pm$  174.70) ng/g for 5a and 5b EIA, respectively. Peak concentrations ranged from 132.22 to 470.31 (Mean  $\pm$  SEM: 311.34  $\pm$  56.85) ng/g and from 2739.33 to 6635.860 (Mean  $\pm$  SEM: 4785.61  $\pm$  634.53) for 5a and 5b EIA, respectively. Following the GnRH injection, FPM peak concentrations for 5a and 5b EIAs were reached 11 days post injection, reflecting an increase of 301 – 1298 % (mean: 711 %) and 321 – 1394 % (mean: 650 %) above baseline levels for 5a and 5b EIA, respectively. Conclusively, serum P4 level was well reflected by FPM concentrations and this can be used as a successful non-invasive tool for monitoring of luteal activity in dromedary.

## Polymorphism in candidate genes related to personality and sociality among three zebra species

ITO HIDEYUKI<sup>1,2</sup>, OGDEN ROB<sup>3</sup>, LANGENHORST TANYA<sup>4</sup>, MURAYAMA-INOUE MIHO<sup>1,5</sup>

<sup>1</sup>Kyoto University, Wildlife Research Center, Kyoto, 2-24 Tanaka-Sekiden-cho, Sakyo, Kyoto, 606-8203, JAPAN; itohide7@gmail.com

<sup>2</sup>Kyoto City Zoo, Okazaki Park, Okazaki Houshoji-cho, Sakyo, Kyoto, 606-8333, JAPAN

<sup>3</sup>Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh, Easter Bush Campus, EH25 9RG, Edinburgh, UK

<sup>4</sup>Marwell Wildlife, Thompsons Lane, Winchester, Hampshire SO21 1JH, UK

<sup>5</sup>National Institute for Environmental Studies, Wildlife Genome Collaborative Research Group, 16-2 Onogawa, Tsukuba, Ibaraki, 305-8506, JAPAN

Equidae is comprised of eight species, which all belong to one single genus, *Equus*. *Equus* contains four sub-genera, *Equus*, *Asinus*, *Dolichohippus*, and *Hippotigris*. Members of the genus *Equus* show two patterns of social structure (RUBENSTEIN, 2011). In the sub-genus *Equus* and *Hippotigris*, a single breeding male with a fixed group of one to six unrelated females and their offspring is observed; this is a harem. On the other hand, in the sub-genus *Asinus* and *Dolichohippus*, breeding males have territories and females do not form stable groups. The diversity of these social structures is not consistent with the genetic phylogeny.

Together with other factors such as ecology and phylogeny, the social structure affects or reflects species differences in personality, such as levels of aggression, affiliation, and pair-bonding. Given that the genus *Equus* displays different social systems, it is a suitable system to investigate the function of personality- and sociality-related genes in different sub-genera. Genetic polymorphisms in genes related to neurotransmitters or hormones (e.g. androgen, oxytocin, arginine vasopressin, and dopamine) affect personality or behavioural traits in many animal species including humans. Previously, we found that there were androgen receptor gene polymorphisms in intra/inter zebra species (ITO *et al.*, 2015). Here, we investigated four genes of neurotransmitters or hormones (serotonin receptor, arginine vasopressin, dopamine receptor, oxytocin receptor) in three zebra species. We found polymorphisms (indels and SNPs) within and between three species at all four genes. These genes represent an important set of candidate genes to study the basis for social systems and behavioural differences, therefore variation in these genes may influence personality and sociality in equid species. Comparison of these genes might help us to understand the evolution of social systems of Equids.

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## Curious lipids in kangaroo sperm and their bad cryoperformance

JAKOP ULRIKE<sup>1</sup>, MÜLLER KARIN<sup>1</sup>, SCHILLER JÜRGEN<sup>2</sup>, ENGEL KATHRIN<sup>2</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Reproduction Biology, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; jakop@izw-berlin.de

<sup>2</sup>University of Leipzig, Institute of Medical Physics and Biophysics, Härtelstr. 16-18, Leipzig, GERMANY

Nearly 20 % of the 67 species from the Macropodidae family including kangaroos are classified as endangered or critically endangered on the IUCN red list; four species were already extinct by 2016. Assisted reproduction techniques become increasingly important for the conservation of endangered species, including sperm cryopreservation. So far no promising cryopreservation protocol has been established for kangaroo sperm (COMIZZOLI P, 2015). Own attempts on kangaroo sperm cryopreservation resulted in immotile sperm after thawing as well.

One determinant of sperm cryoperformance is the species-specific membrane lipid composition (WHITE, 1993), controlling its physical properties. Here, we studied the sperm lipid composition in four different kangaroo species. Since severe morphological changes occur during epididymal maturation of marsupial sperm we also analysed potential differences between caput, corpus and cauda sperm.

By MALDI-TOF MS, ESI IT MS, tandem mass spectrometry and GC MS we found lipids with an uncommon fatty acid (docosatrienoic acid, 22:3), so far unknown in other mammalian sperm. PC 18:0<sub>alkyl</sub>/22:3<sub>acyl</sub> could be detected as the main constituent of cauda sperm (ENGEL, 2017).

First analyses of the sperm lipids before and after cryopreservation attempts indicated a tendency of decrease in saturated fatty acids and MUFAs after thawing. Further investigations will show, if supplementation with particular fatty acids can maintain sperm viability during cryopreservation. Since lipid metabolism remains active in post testicular sperm (SVETLICHNYY *et al.*, 2014), supplementation with selected fatty acids may support repair mechanisms for degraded membrane lipids.

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## **Detecting of Asian and Chinese longhorn beetle (ALB/CLB) by wildlife detection dogs in European quarantine areas**

JENSEN ANGELIKA

Wildlife Detection Dogs e.V., [www.wildlifedetectiondogs.org](http://www.wildlifedetectiondogs.org), Sofienstraße 5, 74867 Neunkirchen, GERMANY

The Asian longhorned beetle (*Anoplophora glabripennis* Motsch, ALB) and the Chinese longhorned beetle (*Anoplophora chinensis*, CLB) were brought to Europe from Asia and belong to the ten most dangerously invasive beetle species. ALB & CLB invade a large variety of trees; the top five host genera infested, in decreasing order, are maple, birch, willow, chestnut and poplar. The EU has strict rules for quarantine, such as a mandatory removal of all potential host trees with a stem of more than 1 cm circumference in a radius of 100 m around the tree that has been found to contain a proof of ALB or CLB in any active status. All potential host trees within the quarantined area must be removed, chipped in place and burnt immediately. The quarantine period of the cleared area lasts for four years but can be extended by another four years in case of a detection of a living ALB or CLB within the quarantine period. Additionally, it is not allowed to remove plants from the quarantine area for either personal or commercial purpose for two years. In the USA, Canada, Austria, The Netherlands, Switzerland, Great Britain and Bavaria, there has been success in removing the quarantine status in some areas encouraged by the continued successful work of wildlife detection dogs in the early detection of ALB/CLB infestation. These dogs are trained to detect the scent of frass, egg laying sites (even old and deceased ones), larvae in all eleven stages of development, pupae and imagos (the beetle itself). They alert with a very high rate of reliability to the presence of the scent of any of the above in the area of roots and stem.

## The consequences of snare injuries on fitness in wild female spotted hyenas

KAIDATZI SARA<sup>1,2</sup>, EAST MARION<sup>2</sup>, HOFER HERIBERT<sup>2</sup>, BENHAIEM SARAH<sup>2</sup>

<sup>1</sup>Free University, Department of Biology, Königin-Luise-Str 12-16, 14195, Berlin, GERMANY; sarakaidatzi@gmail.com

<sup>2</sup>Leibniz Institute for Zoo and Wildlife Research, Dept. of Evolutionary Ecology, Alfred-Kowalke-Straße 17, 10315, Berlin, GERMANY

The consumption of wildlife species by humans is a key threat to the viability of wildlife populations and species. When the hunting methods employed are not specific for the target species, by-catch can pose a threat to non-target species. In the Serengeti National Park (Serengeti NP) in Tanzania, bushmeat hunters set wire snares, mostly in western and north-western areas of the Park, to capture chiefly migratory herbivores. This illegal and unselective hunting technique kills a considerable number of non-target species, including the spotted hyena (*Crocuta crocuta*). Spotted hyenas that 'escape' from a snare do so by biting through the tethering wire. Hyenas that escape from snares are wounded, often have the wires embedded in their flesh and associated serious septic wounds. Some hyenas escape with serious mutilations, for example, when a snare cuts-off or mutilates a foot thereby impeding locomotion, or a deeply incised snare around the neck cuts through the trachea, hampering breathing, eating or drinking. In the Serengeti NP, spotted hyenas are particularly prone to being snared because they forage year round on migratory herbivores. This is particularly the case for females below median social status. Our study aimed to assess whether females of low social status are more prone to exhibit wounds caused by snares. We assess, following the escape of a lactating mother from a snare, whether or not the chance of survival to adulthood of her dependent cubs is reduced. We also compare the lifetime reproductive success of females with and without snare wounds to determine the long-term fitness consequences of surviving a snare in females above and below median rank. Data for the study were obtained from 1987 to 2016 by an on-going long-term study based on a large population of individually known hyenas. Our analysis includes 47 known females. To our knowledge, very few studies have looked at the fitness consequences of injuries caused by wire snares, in relation to individual characteristics, such as social status or age.

## **Increased efficiency and reliability of detecting fish otter (*Lutra lutra*) records by using scat detection dogs?**

KARP DENISE<sup>1,3</sup>, MAUSBACH JELENA<sup>2,3</sup>

<sup>1</sup>University of Zurich, Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, SWITZERLAND; denise.karp@ieu.uzh.ch

<sup>2</sup>ETH Zürich, Eawag, Überland Straße 133, 8600 Dübendorf, SWITZERLAND

<sup>3</sup>Wildlife Detection Dogs e.V., www.wildlifedetectiondogs.org, Sofienstraße 5, 74867 Neunkirchen, GERMANY

The Eurasian otter (*Lutra lutra*) is about to naturally recolonise Switzerland. For the purpose of research, conservation and management, the dispersal of this endangered species should be closely monitored. Eurasian otters are cryptic animals that can hardly be observed. Commonly, otters are documented by searching for scat. When density of otters is low, their marking behaviour is strongly reduced making it very hard to document their presence. Where fish otters are about to recolonise new habitats, density is always small and thus, recolonisation events are very hard to pick up. However, detection of scats can be greatly enhanced by using scat detection dogs. We would like to investigate whether dogs are able to find otter scats more efficient and more reliable than humans can do. For this purpose, the same areas will be searched once by a human expert and once by scat detection dogs. The aim of this study is to evaluate whether it would be reasonable to use scat detection dogs for future Eurasian otter monitoring in Switzerland.

## **The social behaviour of neutered male and female dogs compared to intact dogs (*Canis lupus familiaris*) as a model – video analyses, questionnaires and case studies**

KAUFMANN CARINA ANNA<sup>1,2,3</sup>, LORENZ KRISTIN P<sup>2,3,4</sup>, GANSLOBER UDO<sup>2,3</sup>

<sup>1</sup>Department of Zoology, School of Biology/Chemistry, Institute for Behavioural Biology, University of Osnabrück, Barbarastraße 7, 49076 Osnabrück, Lower Saxony, GERMANY; ckaufman@uni-osnabrueck.de

<sup>2</sup>Zoological Institute and Museum, Johann-Sebastian-Bach-Straße 11/12, 17489 Greifswald, GERMANY

<sup>3</sup>Institut für Spezielle Zoologie und Evolutionsbiologie, mit Phyletischem Museum Erbertstr. 1. 07743 Jena, GERMANY

<sup>4</sup>Department of Animal Ecology and Systematics, University of Giessen, Ludwigstrasse 23, 35390 Giessen, GERMANY

The castration of domestic dogs can be used as a model for reproductive control and captive breeding in zoo canids. Especially, aggressive and attachment behaviours are often based on other mechanisms than on sex hormones. Whereas aggressive behaviour often seems to be stress-related, attachment behaviour is socially motivated and not sexual. Furthermore, group order in packs is not based just on sexual competition, but on leadership quality.

The question to be answered is whether there are differences in certain behaviours between neutered and intact dogs. Using video recordings of twelve groups of dogs, consisting of 18 intact and 16 neutered males and 17 intact and 17 neutered females in total, the social behaviour of the dogs was analysed. Specific behaviours were compared between the castrated and intact dogs and statistically analysed. Furthermore, personality questionnaires based on the studies conducted by TURCSÁN *et al.* (2011) on 294 dogs (n = 104 males, 190 females) and their dog owners have been incorporated into this study. Additionally, 234 case studies (n = 54 males, 180 females) from our consultancy were evaluated.

Significant differences in male dogs were found in patterns of behaviour such as smelling and licking the genital area, chin rest, tooth chatter and molesting other dogs (Mann-Whitney-U-Test,  $U = 382.000$ ,  $p = 0.001$ , when analysed in frequencies; Randomization test for ‘molesting’,  $F = 13.044$ ;  $p = 0.001$ , when analysed for its duration).

While the intact females have shown significantly more social behaviours ( $F = 6.463$ ,  $p = 0.0122$ ), more individual behaviour can be observed for the spayed females ( $F = 41.904$ ,  $p < 0.0005$ ). Moreover, the castrated female dogs show more threatening behaviour ( $F = 6.261$ ,  $p = 0.015$ ) and play less frequently than the intact dogs ( $F = 21,276$ ,  $p < 0.0005$ ).

Finally, our results show a tendency for more aggressive and uncertain behaviour in gonadectomised dogs.

So for contraception in zoo canids it is a concern to examine if there are similar results in wild canids. For future research, personality questionnaires and video recordings from as many as neutered and intact canids are needed with, if possible, determination of stress hormones.

TURCSÁN B, KUBINYI E, MIKLÓSI Á (2011): Trainability and boldness traits differ between dog breed clusters based on conventional breed categories and genetic relatedness. *Appl Anim Behav Sci* **132**, 61-70.

## **Distribution of striped hyenas (*Hyaena hyaena*) in Deukhuri Valley, Nepal**

KHANAL CHIRANJEEVI<sup>123</sup>, BANIYA SANJEEV<sup>3</sup>

<sup>1</sup>Friends of Nature (FON) Nepal, Tinkune, Kathmandu, NEPAL;  
chiran\_khanal2011@hotmail.com

<sup>2</sup>IUCN SSC Hyaena Specialist Group

<sup>3</sup>Institute of Forestry, Pokhara Campus, Hariyo Kharka, Pokhara-15-Kaski District, NEPAL

The striped hyena (*Hyaena hyaena*) is classified as “Near threatened” on the IUCN Red list of threatened species. In Nepal, it has been assessed as nationally endangered with estimated populations fewer than 100 individuals. However, very little information is available about its status, distribution and ecology. Persecution, decrease in natural and domestic source of carrion are the foremost threats for the species. Because of the necessity to investigate this species, this study was carried out in Deukhuri valley which lies in Dang district of west lowland, Nepal. The study was conducted in 10 village development committees (VDCs) by dividing the whole study area of the valley into grids of 5\*5 km<sup>2</sup>, placing one camera trap in each grid followed by sign survey. Hyenas were recorded with camera traps in Satbariya, Chailahi, Sonpur, Sisahaniya, Lalmatiya and Gobardiya VDCs while signs like pugmark of hyena were recorded from all ten VDCs of the Valley. The result of this research depicts that hyenas are distributed throughout the valley, thus the valley retains suitable habitat for hyenas. Very few studies have been conducted on this species and our findings may provide some useful information for its conservation in Nepal and elsewhere.

## Using high-resolution movement data to derive diurnal behavioural patterns of red foxes (*Vulpes vulpes*) along an urbanisation gradient

KIMMIG SOPHIA, GRAS PIERRE, BOERNER KONSTANTIN, BERGER ANNE, BRANDT MIRIAM, HOFER HERIBERT, ORTMANN SYLVIA, KRAMER-SCHADT STEPHANIE

Leibniz Institute for Zoo and Wildlife Research (IZW), Dept. of Evolutionary Ecology, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; kimmig@izw-berlin.de

The expansion of urban areas is one out of manifold aspects of continuously increasing anthropogenic pressures on the environment. More than half of the world's population is already living in urban agglomerations; and the proportion of urban population is forecasted to increase further. This urbanisation process creates challenges for wild animal species, because they have to constantly trade-off their needs versus risks imposed by humans to be able to survive and persist in such novel environments. Many species suffer from these anthropogenic influences; however, some generalist species like the red fox (*Vulpes vulpes*) can cope well and even reach high densities in the urban habitat. It therefore serves as an excellent model species to assess behavioural adjustments to anthropogenic pressures through space-use and movement analyses. The movement pattern of an individual is a reflection of the ecological constraints that it is exposed to and the evolutionary processes that it is subject to. Studying animal movement, therefore, is essential to understand adjustments of animal behaviour to novel habitats such as urban areas.

We here analyse the influence of urbanization on circadian movement patterns of urban red foxes. We hypothesize that human day- and night-time activities influence red fox behaviour. Respectively, we expect to find different circadian movement patterns in areas with varying degrees of urbanisation. We predict that movement speed is higher during diurnal than during nocturnal activity, especially in areas with high levels of urbanisation. We further expect home ranges to be smaller during the day and larger during the night.

To this end, we radio-collared foxes with GPS tags within the city of Berlin. We used high resolution GPS data (4 min intervals) of four individuals, sampled over six to eight weeks, to analyse speed and turning angles with respect to daytime. The degree of urbanisation was estimated based on the percentage of sealed area. We determine the home range sizes of foxes both, diurnal and nocturnal, using GPS data of six individuals applying *minimum convex polygon* (MCP) method, *kernel density estimation* (KDE) and a recently proposed auto-correlated kernel density estimator (aKDE).

Our analyses demonstrate that foxes adjust their movement pattern in response to the circadian change in human activity and reveal the plastic behavioural capacity of a successful mesopredator. This finding has important implications not only for the management of wildlife in urban areas, but also for elucidating the role anthropogenic pressure has as external driver on urban wildlife.

## **Effect of age and pair-mate change on vocal duets of endangered Siberian crane (*Grus leucogeranus*)**

KLENOVA ANNA<sup>1</sup>, GONCHAROVA MARIA<sup>2</sup>, LUKYANCHUK OXANA<sup>1</sup>, KASHENTSEVA TATIANA<sup>3</sup>

<sup>1</sup>Lomonosov Moscow State University, Faculty of Biology, Department of Vertebrate Zoology, Vorobievsky Gory, 1/12, 119234, Moscow, RUSSIA; anna.v.klenova@gmail.com

<sup>2</sup>National Research Center for Hematology, Laboratory of genetic engineering, Novy Zykovsky pr. 4a, 125167, Moscow, RUSSIA

<sup>3</sup>Oka Crane Breeding Center, Oka State Nature Biosphere Reserve, 391072, Brykin Bor, Ryazan Region, RUSSIA

Vocal-based monitoring has proved its use in many animal species and is promising for the Siberian crane. It is a long-lived, slowly reproduced bird with an estimated population of 3500 cranes and is listed as Critically Endangered by the IUCN Red List. Loud duets of nesting pairs could be recorded properly from a distance 700 m in nature and are potentially appropriate for monitoring. Previous findings showed the high pair-specificity of the duets, however it is still unknown how stable they are over a bird's life and what happens with them when the bird changes its mate. Here we studied the stability of duet acoustic variables through the 10 years in 10 reproductively active pairs, whose age at the beginning of the study was 3-26 years. The duets of each pair were recorded in Oka Crane Breeding Center in two periods with the minimum interval of 10 years: in May-July 2003-2005 and 2013-2015. In May-July 2003-2017 with the interval of 3-4 years we also recorded duets of 3 birds (1 male and 2 females) with different pair mates. We measured 18 time-frequency variables in 249 duets (4-12 duets per pair, per period). We did not find general effect of age on acoustic variables of Siberian crane duets (repeated measures ANOVA,  $p > 0.05$  for all variables). Pair-specific vocal signatures remain stable over the period of study. Discriminant analysis showed 100 % percentages of correct classification to pair in each period. And cross-validation of duets from the test sets (represented by samples of 2013-2015) with discriminant functions derived from the training sets (represented by samples of 2003-2005) showed comparable high percentage of correct classification to pair (88.1 %). However, through the study period 9 birds (5 males and 4 females) from 20 studied ones slightly but significantly decreased the fundamental frequencies of their calls and 3 (3 males) from 20 – slightly but significantly changed the temporal variables of their calls (t-test,  $p < 0.003$ ). After mate change the male did not change significantly any of the acoustic variables, one of the female slightly but significantly increased the duration of the calls, another female – slightly but significantly decreased the fundamental frequency of the calls in duets (t-test,  $p < 0.003$ ). But these changes have not exceeded those of duets recorded from birds with one mate over the study period. Our data suggest that Siberian cranes can be reliably identified by their duet parts over a whole bird's life.

Supported by the Russian Science Foundation (grant 14-14-00237).

## **Dynamic of fox cubs' 24-h activity in the wild: from den period to the beginning of dispersion**

KORENKOVA ANNA A<sup>1</sup>, YACHMENNIKOVA ANNA A<sup>2,3</sup>

<sup>1</sup>Lomonosov Moscow State University, Leninskie Gory, Moscow, 119991, RUSSIA; korenkovaa@mail.ru

<sup>2</sup>Severtsov Institute of Ecology and Evolution, Russian Academy of Science, Leninsky prosp. 33, Moscow, 119071, RUSSIA

<sup>3</sup>Kronotsky Nature Reserve, Ryabikova Street, 48, Yelizovo, Kamchatka region, 684000, RUSSIA

Circadian rhythms are cyclical fluctuations of the intensity of various biological processes with a period about 20 - 28h. This internal genetically fixed endogenous rhythm was developed as an evolutionary response to periodic daily changes of parameters of environment. Features of circadian activity are important for all periodic phenomena in the behaviour of any species, and are basic for understanding the rhythmically organized forms of behaviour, which play a crucial role in the behaviour development. Predators' daily rhythm of activity in the wild depends on diet, food availability, circadian rhythm activity of prey, the success of hunting, social structure. Activity of animals which live in family groups is connected. We observed fox (*Vulpes vulpes*) cubs in the wild (June-August 2013, 2014, 2015) in Kronotsky Nature Reserve, MAB, UNESCO, Kamchatka, Russia by using camera traps established on the den sites (3 families,  $N_{\text{cubs}} = 11$ ; 286 cameratrap-days) and analysed database with the Oriana 2.0 software. Changes in the daily activity of cubs at the age 3-13 weeks on the den-site in accordance with their development and identified the time maximum intensity for different types of activities are the next. Peak of pups' activity (except 3<sup>rd</sup> week) is detected for the evening and night hours, while the peak of parents' activity is at 5-8 p.m., except for 12<sup>th</sup> weeks age of pups. The parents peaks of social activity are changing with pups development and don't have an exact trend or coincidence with the peaks of any cubs activity. The intensity of these peaks tends to be statistically significant (Rayleigh Test,  $p < 0,01$ ) when cubs are fed with milk (8<sup>th</sup> week). Confidence intervals for the peaks of overall (social and play) cubs activity on the den-site are overlapped for most ages we analysed. At the age-period when cubs use solid food there is no clear dynamics of intensity of their contacts with parents. In the period of milk dependence there are also no correlation between peaks of parents and any cubs' activities. The peaks of play activity doesn't follow the peaks of the feeding-time. Autogrooming appears on the 4<sup>th</sup> week age and has a statistically significant temporary peak for most of ages (Rayleigh Test,  $p < 0,01$ ). The intensity of evening and night dominates the morning and afternoon, but peaks of different types of activity aren't clearly connected with each other.

Financially supported by Kronotsky State Nature Reserve and RFBR grant №13-04-00192a.

## Multiple hybridisation events in the history of pig domestication compared to wild boars

KASTRIOT KORRO<sup>1</sup>, ŠPREM NIKICA<sup>2</sup>

<sup>1</sup>Department of Veterinary Public Health, Faculty of Veterinary Medicine of Tirana, Agricultural University Kamez Tirana, 1000, ALBANIA; kkorro@gmail.com

<sup>2</sup>University of Zagreb, Faculty of Agriculture, Department of Fisheries, Beekeeping, Game Management and Special Zoology, Svetosimunska 25, 10010 Zagreb, CROATIA

During 2013 and 2014, a 348 bp fragment has been analysed of the control region of mtDNA of 60 domestic pigs and 95 wild boars from Croatia and Albania (SPREM *et al.*, 2014). Thirteen haplotypes were determined in the mitochondrial sequences of Croatian indigenous pigs and in wild pigs from Albania and Croatia. Black Slavonian pigs shared one haplotype with Mangalica and two haplotypes are known from Berkshire which confirms the origin of Black Slavonian pigs by crossing the breeds Mangalica and Berkshire. Black Slavonian, Turopolje, Albanian and Croatian wild boars have in common three haplotypes, while wild boars from Albania and Croatia share only one haplotype. The domestic breed of Black Slavonian, Turopolje and the wild boars from Albania have one specific haplotype. Black Slavonian shared only one haplotype with Turopolje. Croatian wild boars are characterised by additional three haplotypes which they share with other domestic breeds and European wild boars

The analysis confirmed multiple hybridisation and introgression events in the history of pig domestication.

ŠPREM N, MAJA B, MARIJA D, DRAŽEN I, DOMAGOJ D, KASTRIOT K, VLATKA CC (2014): Mitochondrial DNA diversity of domesticated endangered pig breeds and wild boars. 10th International Symposium on Wild Boar and Other Suids, Velenje, Slovenia, 1-5 September 2014.

## **The mRNA expression of receptors for estradiol and enzymes involved in steroid production in the ovary and endometrium of uterus in pre-pubertal, pubertal and sexually mature wild boar sows**

KORZEKWA ANNA<sup>1</sup>, ZADROGA ANNA<sup>1</sup>, ZALEWSKI DARIUSZ<sup>2</sup>

<sup>1</sup>Department of Biodiversity Protection, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Tuwima St. 10, 10-747 Olsztyn, POLAND; a.korzekwa@pan.olsztyn.pl

<sup>2</sup>Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Oczapowskiego St. 2, 10-718 Olsztyn, POLAND

The continuous increase of wild boar population density is observed in Poland, even if it has been stable during the last five years (about 270 thousands). One of the reasons is too early puberty of sows, which may be caused by feed supplementation with zearalenone found in maize. Nowadays, there are observed pregnancies twice a year in wild boar sows and pigs of the sounder become pregnant below two years of age. Puberty is reached by sufficient production of steroids in the reproductive tract. We focused on determination estradiol alpha receptor (ER) and enzymes: 3-beta-hydroxysteroid dehydrogenase (3BHSD) – enzyme involved in progesterone synthesis and aromatase responsible for estradiol production.

The aim: comparison of mRNA expression for factors important for puberty between wild boar sows of different weight for recognizing the weight when boars are ready to begin reproduction.

The experimental material were the reproductive tracts of wild boar sows collected in the area of Warmia and Mazury District of Polish Hunting Association (North-East of Poland) from December 2016 to January 2017. The sows were divided into groups: squeakers – pre-pubertal (15 - 30 kg of body mass, n = 5), pigs of the sounder - pubertal (35 - 70 kg, n = 5) and mature sows (more than 70 kg, n = 5). The mRNA expressions for ER and the enzymes 3BHSD and aromatase were evaluated in tissue of endometrium (horn of uterus adjacent to ovary) and ovary (homogenous tissue without follicles and corpus luteum) by real time PCR.

3BHSD mRNA expression was not observed in squeakers but was on the same level in the two others groups: pigs of the sounder and mature, both in the ovary and endometrium ( $p > 0.05$ ). ER mRNA expression was the lowest in squeakers and the highest in mature sows in ovary ( $p < 0.05$ ). Aromatase mRNA expression was not observed in the youngest group neither in endometrium nor in ovary, in endometrium it was expressed on the same level in the two others groups ( $p > 0.05$ ), in ovary it was lower expressed in pigs of the sounder than in mature sows ( $p < 0.05$ ).

The results indicate that wild sows begin to produce steroids in ovary and endometrium when their weight is in the range defined as the weight of pre-pubertal sows (15 - 30 kg) in Warmia and Mazury (Poland). Further studies are necessary, especially in the wild pigs of the sounder to elucidate the molecular mechanisms, reasons and consequences of such earlier maturation for reproduction are.

## Whom do I select for mating? A study of female mate choice in white rhinos (*Ceratotherium simum simum*)

KRETZSCHMAR PETRA<sup>1</sup>, AULD HAILIE<sup>3</sup>, BOAG PETER<sup>3</sup>, COURTIOL ALEXANDRE<sup>1</sup>, GANSLOBER UDO<sup>2</sup>, GRAS PIERRE<sup>1</sup>, SCOTT CANDACE<sup>3</sup>, WONG PAMELA<sup>3</sup>, VAN COEVERDEN DE GROOT PETER<sup>3</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Dept. of Evolutionary Ecology, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; kretzschmar@izw-berlin.de

<sup>2</sup>Ernst Moritz Arndt University Greifswald, Zoological Institute and Museum, Johann-Sebastian-Bach-Str. 11-12, 17489 Greifswald, GERMANY

<sup>3</sup>Queen's University, Biology, 116 Barrie Street, Kingston, Ontario, CANADA

Female southern white rhinoceros (*Ceratotherium simum simum*) invest a lot of resources in their offspring. After a gestation period of 16 months, they produce a juvenile of 65 kg in weight which they nurse for up to 18 months (PLAYER and FEELY, 1960; OWEN-SMITH, 1973, 1975). Males hardly provide any resources. They join a receptive female about two weeks before mating and leave her a few days afterwards (OWEN-SMITH, 1973, 1975, 1988). Differences in resource allocation occur predominantly in species that display female mate choice (TRIVERS, 1972), the southern white rhinoceros is thus an ideal candidate for a study on reproduction preferences. We combined 11 years of field observation with genetic analysis of fatherhood in order to identify differences in reproductive success among two cohorts of dominant adult male white rhinos (n = 12). Data were collected on a 300 km<sup>2</sup> privately owned game farm in the Limpopo Province in South Africa. The males were described by their morphological characteristics as well as by the size and the quality of their territories. A total of 148 tissue samples were genotyped. Parentage was assigned to 106 mother-offspring pairs based on categorical allocation in Cervus 3.0 and reconstruction using Colony 2.0. Assignments were made at a confidence level of 80 % or higher. We used a principle component analysis to convert correlated male traits into uncorrelated variables and tested paternity using Spearman rank correlation. The outcome of the study will help to prevent further loss of variation through genetic drift or inbreeding, especially in small populations such as on private game farms.

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## **A new food resource for *Glossophaga soricina* (Mammalia: Chiroptera) in Southeast Brazil**

KRUSZYNSKI CECILIA<sup>1</sup>, DINIZ-REIS THAIS ROVERE<sup>1</sup>, PEDROSO AYESHA RIBEIRO<sup>2</sup>

<sup>1</sup>Universidade de São Paulo, Centro de Energia Nuclear na Agricultura, Av. Centenário, 303, postal code 13416-000, Piracicaba (SP), BRAZIL; ceciliak.assis@gmail.com

<sup>2</sup>Universidade Estadual do Rio de Janeiro, Laboratório de ecologia de mamíferos, Rua São Francisco Xavier, 524, postal code 20550-900, Rio de Janeiro (RJ), BRAZIL

The Pallas's Long-tongued Bat (*Glossophaga soricina*) has morphological and behavioural specializations that allow it to explore other food sources, like fruits and insects, when the nectar availability is low. The diet of bats is historically investigated by direct observation, stomach content, and faecal analysis. These bats were observed licking the exposed receptacle of the mature infructescence of the exotic tree *Pandanus utilis* (common screw pine) at a suburban area in southern Brazil. We investigated their behaviour to feed on the receptacle of fruit by systematic direct observations and if this resource was incorporated by stable isotope analysis of visitors' tissue. We captured 15 individuals on one night for identification and tissue sampling and observed at least 30 foraging in groups on a second night around the same tree. To check the stable isotopic contribution of the screw pine into the bat assimilated diet, we collected one pine infructescence from the canopy, other potential resources such as plants consumed by bats (*Ficus*, *Solanum*, *Cecropia*, and *Piper*) and insects at the surrounding areas. Bats presented two feeding behaviours, hover and landing, for licking the receptacle. Bats' hair and liver presented similar isotopic values, correspondent to a mixture of C3 and C4 carbon sources and a third trophic level. They did not seem to incorporate the screw pine into their tissues, so it is probably being used as a fast energy intake. In this anthropic environment, bats seem to be expanding their ecological niche by adding novel food sources in the diet. For that, further investigations should be done to evaluate the role of additional food resources used by bats on anthropic areas.

## **Torpor as a survival strategy during snow storm induced food shortages in Antarctic storm petrel chicks**

KUEPPER NADJA D<sup>1</sup>, LIBERTELLI MARCELA M<sup>2</sup>, CORIA NÉSTOR<sup>2</sup>, QUILLFELDT PETRA<sup>1</sup>

<sup>1</sup>Justus Liebig University of Gießen, Behavioural Ecology & Ecophysiology group, Department of Animal Ecology & Systematics, Heinrich-Buff-Ring 26-32, 35392 Gießen, GERMANY; nadja.kuepper@bio.uni-giessen.de

<sup>2</sup>Instituto Antártico Argentino, Avenida 25 de Mayo 1150, B1650HML San Martín, Buenos Aires, ARGENTINA

Wilson's storm petrels (*Oceanites oceanicus*) are the smallest marine birds breeding in Antarctica, where events like snow storms often prevent parents from providing their offspring with food on a daily basis. To minimize energy expenses in absence of food, Wilson's storm petrel chicks can reduce their metabolism and body temperature by entering torpor. This strategy is known to slow down development, and in time-restricted breeding environments like Antarctica, this can threaten fledging. Hence, we hypothesized that torpor is avoided in well-fed chicks, but will be used during longer fasting times. Chick development in a breeding colony of Wilson's storm petrels on the South Shetland Islands was monitored daily during three consecutive summers by recording chicks' body mass and temperature, as well as environmental parameters. While most chicks were able to maintain stable body temperatures when not fed for one day, some chicks' body temperatures decreased significantly. Age did not differ between those two groups, but chicks maintaining their active body temperatures had, relative to their ages, higher weights. After several days without food, chicks were frequently found in torpor. Most chicks in torpor were able to survive periods of food shortages, reverse their low body temperatures after the next feeding event, and regain body mass. We conclude that torpor is a strong survival strategy used in times of fasting. However, in future scenarios, which may include more frequent snow storms due to climate change, malnourishment could lead to more frequent use of torpor, which could elongate chicks' development, and put them at risk not to fledge in time to survive.

## Noctule bat migration in Central Europe: patterns & plasticity

LEHNERT LINN S<sup>1</sup>, KRAMER-SCHADT STEPHANIE<sup>1</sup>, TEIGE TOBIAS<sup>2</sup>, HOFFMEISTER UWE<sup>3</sup>, POPA-LISSEANU ANA<sup>4</sup>, BONTADINA FABIO<sup>5</sup>, CIECHANOWSKI MATEUSZ<sup>6</sup>, DECHMANN DINA KN<sup>7</sup>, KRAVCHENKO KSENIIA<sup>1</sup>, PRESETNIK PRIEMOZ<sup>8</sup>, STARRACH MARTIN<sup>9</sup>, STRAUBE MICHAEL<sup>10</sup>, ZOEPHEL ULRICH<sup>11</sup>, VOIGT CHRISTIAN C<sup>1</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; lehnert@izw-berlin.de

<sup>2</sup>Büro für faunistisch-ökologische Fachgutachten, GERMANY

<sup>3</sup>natura - Büro für zoologische und botanische Fachgutachten, GERMANY

<sup>4</sup>Estación Biológica de Doñana, Sevilla, SPAIN

<sup>5</sup>Urban Ecology & Wildlife Research, Zurich, SWITZERLAND

<sup>6</sup>Department of Vertebrate Ecology and Zoology, University of Gdańsk, ul. Wita Stwosza 59, 80-308 Gdańsk, POLAND

<sup>7</sup>Max Planck Institute for Ornithology, Department of Migration and Immuno-ecology, 78315 Radolfzell, GERMANY

<sup>8</sup>Centre for Cartography of Fauna and Flora, Ljubljana office, SLOVENIA

<sup>9</sup>BUND-Kreisgruppe Herford, Laarer Str. 318, 32051 Herford, GERMANY

<sup>10</sup>NABU Kreisverband Heinsberg e.V., Eichenstraße 32, 41844 Wegberg, GERMANY

<sup>11</sup>NABU Heinsberg – AG Fledermausschutz, GERMANY

European bats are currently threatened by a variety of substantial indirect and direct anthropogenic stressors, such as habitat degradation, climate change and mortality events at wind turbines. However, detailed understanding of their migratory behaviour and even the migratory connectivity between summer and wintering habitats, which is essential for designing effective conservation measures, is lacking to date. Here, we used stable hydrogen isotopes as intrinsic geographical markers to shed light on the migration behaviour of more than 1,000 noctule bats (*Nyctalus noctula*) found in hibernacula across Central Europe and to unravel the connectivity between hibernacula and likely places of origin. Specifically, we asked the following questions: How plastic is migration in Central Europe? Which general migration pattern do they follow? How repeatable and thus predictable is the migratory behaviour of individuals? Our study confirmed that noctule bats engage in partial and female-biased migration across central Europe, suggesting strongest migration pressures for individuals of northern populations. Further, we revealed a combination of partial, differential and chain migration patterns which lead to a pronounced mixing of individuals from different source populations in hibernacula. Most noctule bats were consistent in their individual migration strategy over time, i.e. 86 % of hibernating bats could be repeatedly assigned to either long-distant or regional migrants across years. This is consistent with our finding that the between-individual component explained 84 % of the variation in  $\delta^2\text{H}_f$  values according to variance component analysis, suggesting specialized individual migration behaviours and a strong faithfulness of individuals to summer areas. However, we also found indications for facultative migratory behaviours in some bats. Our study demonstrates that considerable numbers of protected migratory bats originating from North-eastern Europe and Russia depend on hibernacula across central Europe, calling for an internationally coordinated conservation management.

## How does castration change the integration of immature Western Lowland gorilla males to their familial group network in captive populations?

LETANG BENOIT<sup>1,2</sup>, RIETKERK FRANK<sup>3</sup>, SZÁNTÓ JÁNOS<sup>4</sup>, MULOT BAPTISTE<sup>5</sup>, BRITTON LISA<sup>6</sup>, TER MEULEN TJERK<sup>7</sup>, GUÉRY JEAN PASCAL<sup>2</sup>, SUEUR CÉDRIC<sup>1</sup>

<sup>1</sup>Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, FRANCE; letang.benoit@gmail.com

<sup>2</sup>Zoological institution La Vallée des singes, Le Gureau, 86700 Romagne, FRANCE

<sup>3</sup>Zoological institution Apenheul, J.C. Wilsaan 21, 7313 HK Apeldoorn, THE NETHERLANDS

<sup>4</sup>Zoological institution Artis Amsterdam Royal zoo, Plantage Kerklaan 38-40, 1018 CZ Amsterdam, THE NETHERLANDS

<sup>5</sup>Zoological institution Zooparc de Beauval, Route du Blanc, 41110 Saint-Aignan-sur-Cher, FRANCE

<sup>6</sup>Zoological Institution Chessington World of Adventures, Leatherhead Rd, Chessington KT9 2NE, UK

<sup>7</sup>Zoological Institution Gaia Zoo, Dentgenbachweg 105, 6468 PG Kerkrade, THE NETHERLANDS

The current European captive population of the western lowland gorilla has successful breeding results and a healthy population development. This species has a harem social structure and the balanced sex ratio at birth inevitably leads to difficulties managing male' overpopulation in captive groups. Bachelor groups have been established in zoos in order to counterbalance this trend over the last two decades, but this solution has been proved to have drawbacks over time due to the recurrent stress and agonistic conflicts recorded. For this reason the European Ex-situ Program (EEP) has proposed castration as an alternative means to manage the longevity and welfare of captive gorilla populations. This proposal assumes that the mature castrated males may remain in their native family group in the presence of the silverback male without risking conflicts over access to females. Following the recommendation by the EEP committee, ten immature males have been castrated in six different institutions. We collected data for affiliative behaviours performed in the proximity of other group' members. The study concerns seventy-five individuals of all age classes and both sexes living in seven captive breeding groups (375 hours of focal animal sampling, five hours per individual). In parallel a total of 200 instantaneous samplings were performed every fifteen minutes in each group (20 samplings per day for 10 days). From these data, the results of ten castrated immature males were compared to ten "intact" immature males (five juveniles and five subadults for each category). The resulting analysis and visualisation of the different social networks provides valuable information about the social integration and organization of immature "intact" males in captivity. Moreover this study reveals that castration has a positive impact on the socialisation process of immature male gorillas making them more cohesive members of their group during maturation. We now plan to model the data recorded during this study to evaluate "Positive" (affiliative) and "Negative" (agonistic and abnormal) behaviours, carry out a subjective personality assessment GBI (GOLD KC and MAPLE TL, 1994; KUHAR *et al.*, 2006), and study the amenities of the institutions housing gorillas, and thus hope to answer to the question of whether castration could be an acceptable tool to regulate the male over population in zoological institutions.

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## **Mammal navigation revisited – New methods involving bats as wildlife model for studies of crucial compass orientation**

LINDECKE OLIVER<sup>1</sup>, HOLLAND RICHARD A<sup>3</sup>, PĒTERSONS GUNĀRS<sup>2</sup>, VOIGT CHRISTIAN C<sup>1</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Evolutionary Ecology Research Group, Alfred-Kowalke-Str. 17, 10315, Berlin, GERMANY; lindecke@izw-berlin.de

<sup>2</sup>Latvia University of Agriculture, Faculty of Veterinary Medicine, K. Helmaņa 8, LV-3004, Jelgava, LATVIA

<sup>3</sup>Bangor University, School of Biological Sciences, Deiniol Rd., Bangor, Gwynedd, LL57 2UW, UK

Every night, millions of bats perform long-distance way finding tasks which encompass different scales, e.g., foraging flights within their home range boundaries or seasonal migrations of over 1,000 kilometres to reach wintering grounds. Yet, how bats and other mammals generally solve the navigational challenge of their movements is far from understood. Furthermore, echolocation, the most famous sense for orientation of bats, serves only as short range sonar below 20 m of distance, and thus is considered not to be the ultimate solution to the question about navigation.

In order to increase the methodological tool box for studying compass orientation, esp. in natural settings, we aimed to develop a circular release box for bats (CRBox) suitable for testing directional behaviour, i.e., flight decisions. We tested a prototype version of the CRBox in two species (*Pipistrellus* spp.) during migration season and observed consistency between individual takeoff orientation and directions of subsequent departure flights.

We consider the CRBox a promising option enabling studies of orientation and navigation in the ecophysiological context of a larger number of species, i.e., bats. The CRBox carries the potential to compare navigation mechanisms between (small) mammal clades, e.g. bats and rodents, if relevant compass cues are identified. Likewise, temporal behavioural syndromes, e.g., migration, can eventually be studied in a mammalian model in comparison to birds. On the one side, the latter has always been restricted in rodents, because these move only over small distances, and lack migratory behaviour. On the other side such studies are so far unpractical in larger mammal migrants of which many are famous for their long-distance movements but are unsuitable for experimental treatments.

## **Captive breeding programmes for endangered species: are we using the full potential?**

LÜDERS IMKE<sup>1</sup>, ZAHMEL JENNIFER<sup>2</sup>, MÜLLER KARIN<sup>2</sup>

<sup>1</sup>Westfälischer Zoologischer Garten Münster, Sentruper Str. 315, 48161 Münster, GERMANY;  
imke.lueders@gmail.com

<sup>2</sup>Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke Str. 17, 10315 Berlin,  
GERMANY

Captive breeding is accepted as an important tool for endangered species recovery and maintenance. Establishing self-sustaining populations in human care is the overall goal, which is only achievable through a network of multiple breeders with set husbandry standards, a coordinated breeding program and the exchange or introduction of genetics. On the other hand, this is limited by individual fertility or compatibility, offspring mortality, number of species holders and funds, amongst others.

A major problem that the authors encountered is the time span between recognizing the failure to breed and addressing this condition. Very often we are simply waiting too long. With new veterinary drugs at hand, the advancement of diagnostic tools and assisted reproductive techniques, options for intervention and counter steering are available, but often underused. For example may a breeding soundness evaluation directly show if there is any pathologic condition responsible for infertility. Furthermore, collection of gametes (oocytes and sperm) from live animals or post-mortem and cryobanking thereof are more and more important and readily available for a number of species as genetic back-up or for direct implementation into breeding programmes.

Genetic resource banking has been named as a measure in the World Association of Zoos and Aquaria (WAZA) Vision and Corporate Strategy Towards 2020 for integrated species conservation. At the same time, the IUCN Conservation Breeding Specialist Group (CBSG) now postulates the One Plan Approach, which recommends the management of ex- and in situ endangered species as one population, rather than separate entities. The recognition of these measures by large organizations underlines the importance and pushes for increased efforts. On the basis of selected examples, the authors want to highlight the need for proactive measures to show that increased efforts must go into breeding animals earlier, taking actions as soon as problems are encountered and investing into gamete banking, especially if animals have not contributed to the genetic pool by natural reproduction.

## **Chronic nitrate enrichment decreases severity and induces protection against an infectious disease**

MACEDA-VEIGA ALBERTO<sup>1</sup>, SMALLBONE WILLOW<sup>2</sup>, CABLE JO<sup>2</sup>

<sup>1</sup>Doñana Biological Station-CSIC, Avda. Américo Vespucio, s/n. ES-41092, SPAIN;  
albertomaceda@gmail.com

<sup>2</sup>Cardiff University, The Sir Martin Evans Building, Museum Ave, Cardiff CF10 3AX,UK

Excessive fertilisation is one of the most pernicious forms of global change resulting in eutrophication. It has major implications for disease control and the conservation of biodiversity. Yet, the direct link between nutrient enrichment and disease remains largely unexplored. Here, we present the first experimental evidence that chronic nitrate enrichment decreases severity and induces protection against an infectious disease. Gyrodactylosis is a widespread ectoparasitic disease of aquatic animals. Specifically, this study shows that nitrate concentrations ranging between 50 and 250 mg NO<sub>3</sub><sup>-</sup>/l reduce infection intensity of *Gyrodactylus turnbulli* in two populations of Trinidadian guppies *Poecilia reticulata* experimentally infected, and that the highest nitrate concentration can even clear the parasites from the fish. This result, if added to the fact that host nitrate pre-exposure altered the fish epidermal structure and reduced parasite intensity, suggests that nitrate protected the host against the disease. Nitrate treatments also caused fish mortality. As we used ecologically relevant nitrate concentrations, and guppies are top-consumers widely used for mosquito bio-control in tropical and often nutrient-enriched waters, our results can have major ecological and social implications. In conclusion, this study advocates reducing nitrate levels, including the relevant legislative thresholds, to protect aquatic biota, even though this may control an ectoparasitic disease.

## New insights into Samango monkey speciation in South Africa

MADISHA THABANG, DALTON DESIRE, LINDEN BIRTHE, WIMBERGER KIRSTEN, NUPEN LISA, TORDIFFE ADRIAN, TAYLOR PETER, JANSEN RAYMOND, KOTZE ANTOINETTE

National Zoological Gardens of South Africa, 232 Boom Street, P.O Box 754, 0001, Pretoria, SOUTH AFRICA; Thabang@nzg.ac.za

The Samango monkey (*Cercopithecus albogularis*) population in South Africa represents the southernmost extent of the range of arboreal guenons in Africa. A major threat to African primates (including South Africa) is the loss of habitat as a consequence of land-use transformation and forest resource demands. The Samango monkey belongs to the highly polytypic *C. nictitans* group which is sometimes divided into two species *C. mitis* and *C. albogularis*. The number of subspecies of *C. albogularis* is also under debate and is based solely on differences in morphology. This study aimed at combining molecular, morphometric and pelage colour data to clarify taxonomic uncertainty as to the number of Samango monkey subspecies, as well as their respective distribution ranges in South Africa. Data generated from body measurements, hair banding pattern analyses (done by eye under a halogen lamp) and genetic assessments (mtDNA and nDNA) supports the two recognised subspecies namely *C. a. labiatus* and *C. a. erythrarchus* and argues for separate conservation management units of the distinct genetic entities. Within *C. a. erythrarchus* two lineages are proposed, namely the coastal Samango monkey populations of *C. a. erythrarchus* and the inland *C. a. schwarzi* populations which inhabit the Soutpansberg Mountains in the Limpopo Province (South Africa). The taxonomy of the inland Magoebaskloof population (Limpopo Province, South Africa) however remains unresolved. This population is highly divergent based on mtDNA sequence data, but relatively uniform genetically (nDNA) and phenotypically (morphometrics and pelage colour) to the Soutpansberg population. Thus, it is unclear whether this population can be recognized as belonging to the *C. a. schwarzi* cluster or is a distinct lineage, or whether its distribution range forms a hybrid zone. It was further observed that the endemic *C. a. labiatus*, which only occurs in fragmented forest patches, had the lowest (unbiased) genetic diversity ( $uHe = 0.38$ ) in comparison to *C. a. erythrarchus* ( $uHe = 0.59$ ) and *C. a. schwarzi* ( $uHe = 0.41$ ). The connectivity of habitat utilized by the *C. a. erythrarchus* lineages is much higher compared to that used by *C. a. labiatus*. For this reason, habitat connectivity plays a major role in the potential for speciation as shown by the observed results for *C. a. schwarzi* and *C. a. erythrarchus*, whereas the endemic *C. a. labiatus* showed lower genetic diversity. Habitat discontinuity will also affect migration between populations by hindering gene flow and contributing to the extinction vortex.

## **Do females that experience more social support produce more sons?**

MALDONADO-CHAPARRO ADRIANA A<sup>1,2,3</sup>, HUBBARD LILAH<sup>1</sup>, YANG WEI JENNY<sup>1</sup>, BLUMSTEIN DANIEL T<sup>1,4</sup>

<sup>1</sup>University of California, Dept. of Ecology and Evolutionary Biology, 621 Young Drive South, Los Angeles, CA 90095-1606, USA; marmots@ucla.edu

<sup>2</sup>Max Planck Institute for Ornithology, Dept. of Collective Behaviour, Am Obstberg 1, 78315, Radolfzell, GERMANY

<sup>3</sup>University of Konstanz, Dept. of Biology, Universitätsstrasse 10, 78457 Konstanz, GERMANY

<sup>4</sup>The Rocky Mountain Biological Laboratory, Box 519, Crested Butte, CO 81224, USA

Litter sex ratio has profound effects on offspring behaviour and survival. A variety of factors influence maternal condition, and these may influence sex ratio. While previous studies have identified the potential importance of social environment in explaining variation in sex ratio, we are aware of no studies that have used formal social network statistics to study the relationship between specific social attributes and sex ratio. We asked whether the network measure embeddedness, the degree to which an individual is integrated in its social group, explains variation in litter sex ratio. We found that neither body condition nor embeddedness influenced litter sex ratio. Our results disproved the idea that maternal condition is the main factor influencing litter sex ratio and also eliminated one social attribute as a potential influencing factor. Other network measures should be further explored to determine what attributes of sociality may be influencing litter sex ratio variation.

## Habitat utilisation by lesser jerboa (*Jaculus jaculus*) in degraded arid land habitats

MATRAH ALMUTAIRI

Environment and Biological Sciences Research Center, Kuwait Institute for Scientific Research, P.O. Box: 24885, Safat 13109, KUWAIT; mmutairy@kISR.edu.kw

Many arid environments have suffered from anthropogenic interference, which has heavily impacted their natural resources and has caused habitat degradation with severe consequences for wild animals (OMAR *et al.*, 2001; MISAK *et al.*, 2002; AL-DOUSARI, 2005). Identification of habitats within arid land which are highly utilized by animals is important, since it allows decision-makers to target habitats worthy of protection and restoration. The effect of habitat utilisation of lesser jerboas (*Jaculus jaculus*) was assessed in intact, recovered and degraded areas in the State of Kuwait. The study was conducted at Kabd area, 30 km<sup>2</sup> from Kuwait city for a period of one year covering different seasons (2015-2016). The abundance of the jerboa was significantly different in intact, recovered and degraded areas ( $F = 12.2$ ,  $df = 2$ ,  $p = 0.02$ ), but there was no effect of season on their abundance ( $F = 0.65$ ,  $df = 2$ ,  $p = 0.68$ ). Selection indices showed that jerboas and other related animals were less abundant in areas where human activities took place, and to a lesser extent in areas covered by sand dunes (AL-MUTAIRI *et al.*, 2012). The profound lack of suitable habitat for lesser jerboas in degraded areas suggests that lesser jerboas in Kuwait are threatened by habitat loss, a threat which demands more research attention.

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## **The use of wildlife detection dogs for bat roost detection**

MAUSBACH JELENA<sup>1,2,3</sup>, PEERENBOOM GEVA<sup>3,4</sup>, TAUBMANN JULIA<sup>3,4</sup>

<sup>1</sup>ETH Zürich, Eawag, Überland Straße 133, 8600 Dübendorf, SWITZERLAND;  
jelena.mausbach@gmx.de

<sup>2</sup>Stiftung Fledermausschutz Schweiz, Zürichbergstraße 221, 8044 Zürich, SWITZERLAND

<sup>3</sup>Wildlife Detection Dogs e.V., www.wildlifedetectiondogs.org, Sofienstraße 5, 74867 Neunkirchen, GERMANY

<sup>4</sup>University of Freiburg, Wildlife Ecology and Management, Tennenbacher Straße 4, 79106 Freiburg; GERMANY

The use of wildlife detection dogs is getting more and more popular for detecting elusive species and its signs in basic research and for monitoring applications. In the case of bats, dogs are not only used for carcass detection in areas with wind power farms, but in recent years attempts have been made to use them for roost detection at buildings and natural shelters as well. Compared to traditional methods such as telemetry which require catching bats, the use of detection dogs seems less invasive and potentially more effective even detecting old roosts through their smell (remains of faces). Our team is currently evaluating the use of detection dogs to detect bat roosts in Germany and Switzerland using collected bat faces samples and known roosts. Pilot experiments using four detection dogs will be conducted testing for species specific detection versus detection of all bat species, the risk of false positive and false negative detection, and detection probability under different sample preparation and environmental conditions (weather, lag time sample distribution until search, age and height of samples). We will present our preliminary results of those pilots and plans for future studies. Applications could be manifold using such bat roost detection dogs for monitoring and research purposes in forests and at buildings, before renovation of buildings or for screening wood logs after forest clearances.

## Can RAD-Seq help sea turtle conservation?

MAZZONI CAMILA<sup>1,2</sup>, SANTOS FABRICIO<sup>3</sup>, JORDÃO JULIANA<sup>4</sup>, CHEVALLIER DAMIEN<sup>5</sup>, DE THOISY BENOIT<sup>6</sup>, TORRES VILACA SIBELLE<sup>1,2,7</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research (IZW), Department of Evolutionary Genetics, Alfred-Kowalke-Strasse 17 10135 Berlin, GERMANY; mazzoni@izw-berlin.de

<sup>2</sup>Berlin Center for Genomics in Biodiversity Research (BeGenDiv), Königin-Luise-Strasse 6-8 14195 Berlin, GERMANY

<sup>3</sup>Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627 31.270-010 Belo Horizonte, MG, BRAZIL

<sup>4</sup>Universidade de São Paulo, Department of Evolutionary Genetics, Rua do Matão 277 Cidade Universitária 05508-090 São Paulo, SP, BRAZIL

<sup>5</sup>French National Center for Scientific Research, 23 rue Becquerel 67087 Strasbourg Cedex 2, FRANCE

<sup>6</sup>Institut Pasteur in French Guiana, 23 Avenue Pasteur, Cayenne, FRENCH GUIANA

<sup>7</sup>Department of Biology, Trent University, 1600 West Bank Drive K9L1Z8 Peterborough, ON, CANADA

From vulnerable to critically endangered, the seven existing sea turtle species have long suffered from anthropogenic actions, such as overhunting/poaching, large-scale fishing activities, pollution and habitat degradation. Different phenomena such as hybridisation and extreme low molecular diversity have been detected for some populations in different studies, but it is unclear how the recent pressures have been involved in changes in the evolutionary history of sea turtles. The amount and diversity of molecular markers available to study such influences is still very low and analyses lack statistical confidence in many cases due to low variation and/or high levels of shared SNPs. We have decided to use one single genomic approach that can boost the number and variety of nuclear molecular markers available for all sea turtle species. Assuming a deceleration of evolutionary rates in sea turtles, we have used the same Double-Digest Restriction Associated DNA (ddRAD) sequencing methodology for the five sea turtle species with worldwide distribution and generated thousands of new molecular markers that will be useful for a large number of important conservational questions, such as levels of population structuring and composition of mixed-stock. The approach we present in this study is a simple but effective solution to largely deepen the knowledge upon ddRAD data produced that can be transferred to virtually any given species or population.

## **The role of the gut microbiota in the herbivore-plant co-evolutionary arms-race**

MENKE SEBASTIAN<sup>1</sup>, FACKELMANN GLORIA<sup>1</sup>, VUCETICH JOHN A<sup>2</sup>, VUCETICH LEAH M<sup>2</sup>, SOMMER SIMONE<sup>1</sup>

<sup>1</sup>University of Ulm, Institute of Evolutionary Ecology and Conservation Genomics, Albert-Einstein Allee 11, 89069, Ulm, GERMANY; sebastian.menke@uni-ulm.de

<sup>2</sup>Michigan Technological University, School of Forest Resources and Environmental Science, 1400 Townsend Drive, Houghton, MI 49931 USA

Although many plants and herbivores established mutualistic relationships, various plant species simply try to avoid being eaten by producing plant secondary compounds (PSC) which can cause illness and even mortality in herbivores. Herbivores, however, are sometimes forced to feed on plants rich in PSCs in times when other plant sources are scarce which raises the question how detoxification of PSCs takes place. Recent research has provided first evidence for the role of the gut microbiota in assisting herbivores' physiological detoxification to overcome the negative effects of PSCs. Moose (*Alces alces*) of the Isle Royale National Park (Michigan, USA), especially during the winter, mainly feed on Balsam fir (*Abies balsamea*), a species of evergreen coniferous trees, which produces PSCs such as terpenes. To investigate if the gut microbiota of this large herbivore plays a role in detoxification of PSCs, we sequenced the faecal metagenome of 71 moose and assigned shotgun metagenomic sequencing reads to bacterial taxonomy and function. We identified bacterial species belonging to genera such as *Novosphingobium*, *Pseudomonas*, and *Rhodococcus* which carry genes encoding for monoterpene transforming enzymes. Thus, our study is among the first to highlight the role of the gut microbiota in detoxification of PSCs in large herbivores driven by the herbivore-plant co-evolutionary arms-race.

## **The chiroptera gut microbiota: evidence for bifidobacteria presence in *Rousettus aegyptiacus***

MODESTO MONICA<sup>1</sup>, SANSOSTI MARIA CRISTINA<sup>1</sup>, PERPETUINI GIORGIA<sup>2</sup>, REGAIOLLI BARBARA<sup>3</sup>, SANDRI CAMILLO<sup>3</sup>, SPIEZIO CATERINA<sup>3</sup>, TOFALO ROSANNA<sup>2</sup>, MATTARELLI PAOLA<sup>1</sup>

<sup>1</sup>Department of Agricultural Sciences, University of Bologna, Viale Giuseppe Fanin, 44, 40127, Bologna, ITALY; paola.mattarelli@unibo.it

<sup>2</sup>Faculty of BioScience and Technology for Food, Agriculture and Environment, Via Spagna, 1, 64023 Mosciano Sant'Angelo, University of Teramo, Teramo, ITALY

<sup>3</sup>Parco Natura Viva - Garda Zoological Park, Loc. Figara, 40, 37012, Bussolengo, ITALY

Bifidobacteria represent one of the main bacterial groups occurring in the gut of primates and other animals and are well known for their positive role on host health. Moreover, *bifidobacteriaceae* are used in coevolution studies since they are maintained within host lineages across hundreds of thousands of host generations. Therefore, the discovery of bifidobacterial diversity in the gastrointestinal tract together with the isolation and characterization of novel bacterial taxa in different hosts is essential. Until now no evidence has been reported about the occurrence of bifidobacteria in bats which are a crucial part of the ecosystem and are ecologically and economically advantageous to humans especially frugivorous ones. This study aimed at the investigation of bifidobacteria in bats belonging to *Rousettus aegyptiacus* species which were born in Egypt and hosted in Natura Viva Garda Zoological Park (Bussolengo, Verona, Italy). Faecal samples were serially diluted and plated on selective medium MRS supplemented with 0.05 % (w/v) cysteine, 100 mg/l norfloxacin, 2 mg/l cycloheximide, 50 mg/l mupirocin and 1 ml/l acetic acid. Strains were typed by BOX-PCR. Representative isolates of each cluster were identified sequencing 16S rRNA gene and studied for phylogenetical relationship with other *Bifidobacteriaceae*. Similarities among profiles were calculated by clustering the Pearson's correlation matrix using UPGMA algorithm. A total of 13 bifidobacteria were isolated and two of them belonged to new bifidobacterial species according to 16S rRNA gene analysis. The majority of strains revealed a phylogenetic relationship with bifidobacteria isolated from non-human primates. This evolutive link of bifidobacterial microbiotas between bats and non-human primates will be further investigated studying bifidobacteria characterizing *Rousettus aegyptiacus* bats from Egypt in order to offer new insights about bats origin.

## Occurrence of Bifidobacteriaceae in herbivorous primate *Theropithecus gelada*

MODESTO MONICA<sup>1</sup>; SANSOSTI MARIA CRISTINA<sup>1</sup>; PERPETUINI GIORGIA<sup>2</sup>; GALLONI DEBORA<sup>1</sup>; POLICANO GIUSEPPINA<sup>1</sup>; LA CAUZA GIUSEPPE FEDERICO<sup>3</sup>; SANDRI CAMILLO<sup>3</sup>; SPIEZIO CATERINA<sup>3</sup>; TOFALO ROSANNA<sup>2</sup>; MATTARELLI PAOLA<sup>1</sup>

<sup>1</sup>Department of Agricultural Sciences, University of Bologna, viale Fanin 44, 40127 Bologna, ITALY; paola.mattarelli@unibo.it

<sup>2</sup>Faculty of BioScience and Technology for Food, Agriculture and Environment, University of Teramo, via Carlo R. Lericci 1, 64023 Mosciano Sant'Angelo (Te), ITALY; rtofalo@unite.it

<sup>3</sup>Natura Viva Garda Zoological Park, Loc. Figara 40, 37012 Bussolengo (Vr), ITALY; spiezio@parconaturaviva.it

Primates in general exhibit a wide dietary diversity, enabling them to occupy various ecological niches. It is well known that the diet greatly influences microbial diversity of gut microbiota and generally, a fibre rich diet promotes the growth of microbial groups producing short fatty acids, thus playing an important beneficial role in the host health. The *Bifidobacteriaceae* are gram-positive, pleomorphically branched, non-motile, non-spore-forming that constitute a significant proportion of the microbiota in the gut. They can be grouped on the basis of one of six different ecological niches that they occupy: the human intestine, oral cavity, food, the animal gastrointestinal tract, the insect intestine, and sewage. Although nearly all primates are to some extent omnivorous, most primates can be primarily categorized as fruit-, insect- or leaf-eaters. However, in all primate diversity, there is only one species of modern primate that feeds on particularly nutrient-poor plant material is the gelada baboon (*Theropithecus gelada*). Native to the highlands of Ethiopia, they subsist largely as grazers, the only modern primate species to do so. Aim of this work was to investigate the occurrence of *Bifidobacteriaceae* in six adult males of *Theropithecus gelada* housed in Parco Natura Viva Garda Zoological Park (Bussolengo, Verona, Italy). Different selective media were tested for their suitability in the isolation of the *Bifidobacteriaceae* strains. All sixteen isolates were clones as resulted from BOX-PCR fingerprinting, a technique allowing the molecular typing. Comparative analysis of the 16S rRNA gene sequences revealed low sequence similarity of isolates to known *Bifidobacteriaceae* and the highest similarity (96.1 %) was found to *Alloscardovia criceti*. Therefore the isolates are phylogenetically not closely related to known species and are possible novel species in the genus *Alloscardovia* of the *Bifidobacteriaceae* family. For the first time member of *Bifidobacteriaceae* family have been found in *Theropithecus gelada* and further studies are needed to better analyse the importance of their presence in these very peculiar group of monkeys.

## Validation of a protocol for corticosterone extraction from feathers of a raptor species

MONCLÚS LAURA, TALLO-PARRA ORIOL, CARBAJAL ANNAIS, LOPEZ-BEJAR MANEL

Universitat Autònoma de Barcelona, Veterinary Faculty, Dept. of Animal Health and Anatomy, Building V, UAB Campus, 08193, Bellaterra (Barcelona); SPAIN; laura.monclus@uab.cat; manel.lopez.bejar@uab.cat

Measuring corticosterone in feathers is a valuable technique that provides a long-term measure of hormone's integration over time. To date, few studies have validated the extraction procedure of corticosterone from feathers (CORTf) and the majority have used the classic protocol described by BORTOLOTTI *et al.* (2008). Here, we provide a new optimized protocol for extracting CORTf in a more timesaving way. We increased the extracting feather area and used less methanol volume. We validated biochemically the new protocol using an enzyme immunoassay (EIA) kit (corticosterone ELISA KIT; Neogen® Corporation) and compared the extracted CORTf levels with the ones obtained from the classic protocol. Three to five interscapular feathers ( $37.1 \pm 2.2$  mm) were sampled from twenty red kites (*Milvus milvus*). After discarding the calamus, feathers were cut into pieces  $< 5$  mm<sup>2</sup> with scissors. We separated 30 mg and placed in a polypropylene tube for the classic protocol and the rest were minced with a ball-mill at 25 Hz for 5 min for obtaining powder. Then, 30 mg of this powder were placed in a new polypropylene tube for the new protocol. For the classic protocol, we added 10 ml of methanol to the cut feathers and vortexed for 30 min, followed by overnight incubation at 32 °C in a shaking water bath. Methanol extracts were separated from feather material using vacuum filtration and the resulting supernatant was placed in an oven at 37 °C until total dryness. For the new protocol, we added 1.5 ml of methanol to the powder and vortexed for 30 min at room temperature, followed by overnight incubation as above. Samples were then centrifuged at 4050 x g at 25 °C for 15 min and 1mL of supernatant was transferred to new aliquot that was placed in an oven at 37°C until total dryness. In both cases, dried extracts were reconstituted with 0.25 ml of EIA buffer, shaken for 1 min, and stored at -20 °C until analysis. After validation assays, the intra-assay coefficient of variation was 5.87 %, the percentage from the spike-and-recovery test was  $113.42 \pm 9.2$  %, the linearity of dilution showed an  $R^2 = 0.99$  with an efficiency of CORTf extraction of  $105.09 \pm 26.3$  %. The standard and pool curves showed parallel displacement ( $R^2 = 0.93$ ,  $p = 0.02$ ). When comparing both protocols, a positive correlation was found between the extracted CORTf levels ( $R^2 = 0.85$ ,  $p < 0.001$ ). According to the results, the new protocol successfully extracts corticosterone from feathers proportionately to the classic protocol, opening the opportunity to work with small sample size and facilitating the methodological procedures.

BORTOLOTTI GR, MARCHANT TA, BLAS J, GERMAN T (2008): Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Funct Ecol* **22**, 494-500.

Acknowledgements: Authors gratefully acknowledge Maria Cortés and other staff from Alfranca Rehabilitation Centre for providing red kites feather samples; and Fundación Tatiana Pérez de Guzmán el Bueno for financial support through a pre-doctoral fellowship to L. Monclús.

## **The wildlife sperm atlas project: documenting morphological diversity**

MUSEKWA TAKALANI<sup>1</sup>, KOTZE ANTOINETTE<sup>1,2</sup>, VAN DER HORST GERHARD<sup>1,3</sup>

<sup>1</sup>Research and Scientific Services Department, National Zoological Gardens of South Africa, P.O. Box 754, Pretoria, 0001, SOUTH AFRICA; takalanim@nzg.ac.za

<sup>2</sup>Department of Genetics, University of the Free State, PO Box 339, Bloemfontein, SOUTH AFRICA

<sup>3</sup>Department of Medical Biosciences, University of the Western Cape, Private Bag X17, Bellville 7530, SOUTH AFRICA

Sperm cells are the most diverse cells in the animal kingdom and are highly differentiated. A wide degree of diversity in sperm morphology is present across species and the ability of spermatozoa to fertilise depends on morphological factors such as shape and size, which affect sperm penetration of the ovum. This project aims to compile a database of sperm characteristics of wildlife species and to provide information on sperm morphology and motility using a computer-assisted sperm analysis system (SCA® CASA). All information is compiled into a sperm database containing monographs for each species to serve as a reference for future research into wildlife reproductive technologies and captive breeding. Each monograph contains information on sperm morphology (e.g. head length/width, head area, acrosome percentage, mid piece length and tail length), sperm motility characteristics (e.g. total and progressive motility), sperm concentration and collection methods. Currently the atlas includes a wide selection of species such as impala, springbok, bottlenose dolphin, lion, rhino, tankwa goat, Arabian oryx, wild dog, hippo and black wildebeest. A majority of sperm samples were collected through epididymal flushes and cryopreserved for up to ten years. Although the extended cryopreservation practices influenced the motility of the sperm samples as expected, valuable monographs were still obtained from sufficient comparative information. Some interesting observations were with springbok having the smallest sperm heads as compared to impala and black wildebeest with no significant differences in the percentage acrosome coverage of the sperm head amongst all three species. We envisage that this baseline information will enhance the value of comparative characteristics between different species and taxa and may aid in future studies focused on reproductive biology, including prediction of the potential fertility.

## The effect of different factors on androgen levels in lynx cubs

NAIDENKO SERGEY, ANTONEVICH ANASTASIYA, EROFEEVA MARIYA

A.N. Severtsov Institute of Ecology and Evolution, Department of Behaviour and Behavioural Ecology of Mammals, Leninsky pr. 33, 119071, Moscow, RUSSIA; snaidenko@mail.ru

Testosterone (T) is one of the main steroids circulating in vertebrates' blood. Similarly to other androgens, T affects animals' sexual and aggressive behaviour (HEMSWORTH and TILBROOK, 2007; EISENEGGER *et al.*, 2011). However, the effects of T were described predominantly for adult animals or for the prenatal period of ontogenesis (DRICKAMER, 1996; HOTCHKISS *et al.*, 2007). The data for changes in androgens during postnatal ontogenesis in Carnivores are limited. We described earlier that T level was at the minimum in Eurasian lynx cubs at the second month of development (NAIDENKO and EROFEEVA, 2005). The aim of this study was to identify the period of maximal concentrations of androgens in lynx ontogenesis and to detect the factors affecting T level (females' individuality, litter size, sex of the cub). This study was conducted at the biological station "Tchernogolovka" near Moscow. We analysed changes in testosterone level during early ontogeny (until three months of age) in 60 Eurasian lynx cubs of 23 different litters. Blood samples (0.05 - 2.50 ml) were collected once per two weeks right after birth and until 3 months of age. Blood was centrifuged for 20 minutes at 1000 G and the serum was stored until the analysis. T and androstenedione concentrations were measured with EIA. Testosterone level was at the maximum in lynx cubs right after birth (in cubs 3 - 4 days old - 1,25 ng/ml), decreased later to the sixth week of age (0,26 ng/ml) and increased again to three months of age (0,6 ng/ml). Sex of the cubs did not affect their testosterone level, but litter size and litter ID had significant effect on cubs' hormonal level. We found no correlation with the hormonal status of the mother (at 15 days of pregnancy). The differences in androgens levels at the 4<sup>th</sup> and 10<sup>th</sup> week of age were related to the litter size. In both case T level was higher in cubs in big litters. In adult females average T increased 1.7 times ( $75 \pm 24\%$ ) over the course of the pregnancy. Probably this increase determines the high level of T in new-born lynx cubs. T peak was described earlier for many mammalian species and it correlates both with the mothers' androgens exposure and embryonal activity of Leidig cells (CLARKSON and HERBISON, 2016). Unlike to many other mammals, T level in lynx cubs remains quite high for two weeks after birth. Thus, T level in lynx cubs change gradually after birth and is not determined by the sex of animals. This study was supported by RFBR grant № 16-04-00757.

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## Recovery of Russian population of Mongolian gazelle

NAIDENKO SERGEY<sup>1</sup>, KIRILYUK VADIM<sup>2</sup>

<sup>1</sup>A.N.Severtsov Institute of Ecology and Evolution, Department of behaviour and behavioural ecology of mammals, Leninsky pr. 33, 119071, Moscow, RUSSIA; snaidenko@mail.ru

<sup>2</sup>Dauriskii Biosphere Nature State Reserve, Komsomolskaya Street 76, 674480, Nizhnii Tsasuchei, Zabaikalskii krai, RUSSIA; vkiriliuk@bk.ru

Mongolian gazelle (*Procapra gutturosa*) is the most numerous antelope species of Eurasian steppes and deserts. This species primarily lives in Mongolia where the number of this gazelle exceeds 1 million individuals. A small number of animals resides in north-eastern China. In Russia, Mongolian gazelle was abundant in the 1950ies and almost disappeared by end of the century. Since 1992, Mongolian gazelle started to reproduce near the Daurian Reserve. Now the number of animals in Russia is close to 8000. Mongolian gazelle is a widely migrating species however, females aggregate with conspecifics in particular areas to give birth. The aim of our study was to compare the body mass of new-born calves and the seroprevalence of foot-and-mouth disease virus of Daurian population of gazelles with three other populations in Mongolia. The study was conducted in 2015 - 2017. We captured 316 new-born (1 - 3 days old) calves in the four populations. The calves usually hide in the grass/bushes and were captured with nets. Calves were weighed, sexed, blood-sampled and hair samples were taken from their shoulders. Animals were released immediately after procedures which lasted on average five minutes. Blood serum was frozen until further analysis. Antibodies against foot-and-mouth disease virus were detected by the enzyme immunoassay kit of LT Biothech (Vilnius, Lithuania). New-born calves of the Daurian population were significantly heavier than in the other three populations and varied from 4.26 kg to 5.05 kg. Weather conditions affected body mass as well because calves body mass was lower during dry hot summers than during colder and more humid one. Larger body mass of new-born calves suggests that females in the Daurian population are in better body conditions than in the other populations. If so, the Trivers-Willard hypothesis predicts that these females should invest more in male offspring. However, the sex ratio of new-born calves was biased towards females in the Daurian population (60 %) compared to the Mongolian ones (42 %, 46 % and 51 %, respectively). Seroprevalence of foot-and-mouth disease virus was significantly lower in the Daurian reserve (5 %) than in the three other populations (21, 25 and 29 %, respectively). The Daurian population is the only one with an increase in the number of individuals in contrast to the three other populations. This population is currently increasing its range, which might correlate with the dry period of the 40-year cycle of the temporary Torey lakes in Russia. These weather conditions may help the Mongolian gazelle to recolonize its former range in Russia.

## Physiological correlates of exposure to avian malaria in a Hawaiian Honeycreeper

NAMES GABRIELLE<sup>1</sup>, SCHULTZ ELIZABETH<sup>2</sup>, KRAUSE JESSE<sup>1</sup>, WINGFIELD JOHN<sup>1</sup>

<sup>1</sup>University of California at Davis, Department of Neurobiology, Physiology, and Behavior, 1 Shields Ave, Davis, California, 95616, USA; grnames@ucdavis.edu

<sup>2</sup>Kenyon College, Department of Biology, 103 College Rd, Gambier, Ohio, 43022, USA

Invasive species are among the greatest contributors to modern animal extinction. In particular, diseases are invading novel habitats and hosts at unprecedented rates. Since the introduction of avian malaria to Hawaii in the early 1900s, it has played a significant role in reducing the abundance and distribution of many native Hawaiian honeycreepers (Drepanidinae), i.e. *Vestiaria coccinea*. The impacts of malaria on honeycreeper populations have been especially significant in low elevation habitats, where the cold-intolerant disease vector (the *Culex* mosquito) is prevalent. Of the remaining 24 honeycreeper species, only the Hawaii Amakihi (*Hemignathus virens*) has maintained stable populations at low elevations. All others are restricted to high elevation (> 1200 m) mosquito-free refugia. Recent studies suggest that resident low elevation Amakihi populations have evolved tolerance to the disease, whereas resident high elevation populations remain susceptible to malaria. The consequences of malaria tolerance and chronic infection on the physiology, behaviour, and survival of low elevation Amakihi, however, remain largely unexplored.

Due to the complex interplay among physiology, immune function, and behaviour, we hypothesise that each of these elements has changed in malaria-exposed Amakihi. In this study, we specifically investigated how exposure to avian malaria affects stress physiology by quantifying plasma corticosterone (CORT) concentrations. While CORT is necessary for the regulation of metabolic processes and mediating the stress response, it can be immunosuppressive. Consequently, avian malaria relapses have been shown to correlate with episodes of chronically elevated CORT. Thus, we predicted that CORT concentrations would be reduced in malaria-exposed individuals so as to increase their immune capacity to fight off infection. We sampled baseline CORT in wild Amakihi in low elevation (malaria-exposed, N = 26) or high elevation (malaria-unexposed, N = 64) habitats. Our results suggest that baseline CORT was consistent across elevations ( $p = 0.80$ ) and does not likely vary with infection status. The relationship between body fat and CORT, however, appears to be related to sampling elevation. We found a significant negative relationship between fat and CORT in low elevation birds ( $p = 0.01$ ) but not in high elevation birds ( $p = 0.25$ ). This suggests that an interaction between disease status and fat stores may have an effect on degree of environmental stress. Given the inevitable spread of malaria with climate change, a comprehensive understanding of the consequences of chronic infection in the Amakihi is critical to determine the management practices needed to protect this and other honeycreeper species.

## **Assessing spatiotemporal interactions between species from camera trapping data**

NIEDBALLA JÜRGEN<sup>1</sup>, WILTING ANDREAS<sup>1</sup>, SOLLMANN RAHEL<sup>2</sup>, HOFER HERIBERT<sup>1</sup>, COURTIOL ALEXANDRE<sup>1</sup>

<sup>1</sup>Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; niedballa@izw-berlin.de

<sup>2</sup>University of California Davis, 1088 Academic Surge, Department of Wildlife, Fish and Conservation Biology, One Shields Avenue, CA 95616, Davis, USA

Understanding spatiotemporal patterns of species interactions is of fundamental interest to behavioural and community ecology. Observer-independent, unbiased methods such as camera trapping facilitate the study of interactions in challenging habitats, but analyses are hampered by a lack of suitable approaches with well-defined properties. We present a flexible and expandable framework to explore and simulate spatiotemporal species interactions which can improve methods to detect spatiotemporal interactions. We simulated species records in a two-species system of a dominant and a subordinate species across a range of activity patterns and interaction strengths. To assess spatiotemporal avoidance, we compared different time intervals between species records using linear models, Mann-Whitney U-test, a permutation test and a test based on randomly generated records. We assessed the statistical power (the ability to detect an existing effect) and robustness (the ability to detect no effect when none is present) of all tests. Power for detecting spatiotemporal avoidance between species was strongly affected by interaction strength and highest for linear models, and reliable above 50 records per species. All tests were robust even at low sample sizes, above a minimum of ten records per species. Linear models were most suitable to analyse spatiotemporal avoidance and can easily correct for confounding effects of other sources of variation in interactions.

## **Costs and benefits of torpor use in times of a warming climate**

NOWACK JULIA<sup>1,2</sup>, STAWSKI CLARE<sup>2</sup>, TARMANN IRIS<sup>1</sup>, GIROUD SYLVAIN<sup>1</sup>, RUF THOMAS<sup>1</sup>, GEISER FRITZ<sup>2</sup>

<sup>1</sup>Research Institute of Wildlife Ecology, Department of Integrative Biology and Evolution, University of Veterinary Medicine Vienna, Savoyenstrasse 1, 1160 Vienna, AUSTRIA; Julia.nowack@vetmeduni.ac.at

<sup>2</sup>Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale 2351, AUSTRALIA

Torpor, the ability of heterothermic animals to reduce metabolic rate and body temperature during challenging periods, has many more functions than just energy conservation in winter. Recent findings in torpor research have revealed that animals employ torpor to survive and cope with adverse and unpredictable conditions during and after natural disasters, in particular fires and storms. Such data support the hypothesis that heterotherms with their flexible energy requirements have an adaptive advantage over homeotherms in response to unpredictable conditions. As climatologists predict that climate change will cause increases to the frequency and intensity of extreme weather events, the opportunistic use of torpor to survive these events will therefore likely enhance survival. However, it is unclear whether torpor use may also impose a risk during such conditions as it may delay perception of stimuli, such as smoke, and because it hinders locomotor performance. Furthermore, obligate hibernators may likely face negative consequences as a result of the predicted increase in surface temperature, since higher winter temperatures are associated with an increase of interbout arousals during hibernation. We will present new data on behaviour and physiology of daily heterotherms, as well as obligate hibernators responding to climatic changes associated with global warming, thereby evaluating risks and advantages of torpor use in the context of climate change.

## Reproductive performance of Scandinavian female brown bears (*Ursus arctos*) in relation to type of hibernation den

NOWACK LINDA<sup>1</sup>, STEYAERT SAM<sup>2,3</sup>, FRIEBE ANDREA<sup>4</sup>, ZEDROSSER ANDREAS<sup>1,3</sup>, SWENSON JON<sup>2,5</sup>

<sup>1</sup>University of Natural Resources and Applied Life Sciences, Vienna, Institute of Wildlife Biology and Game Management, Department of Integrative Biology and Biodiversity Research, Gregor-Mendel-Straße 33, A-1180 Vienna, AUSTRIA; linda.nowack@gmx.de

<sup>2</sup>Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, PO Box 5003, NO-1430 Ås, NORWAY

<sup>3</sup>Faculty of Technology, Natural Sciences and Maritime Sciences, University College of Southeast Norway, NO-3800 Bø i Telemark, NORWAY

<sup>4</sup>Scandinavian Brown Bear Research Project, Tackåsen Kareliusvägen 2, SE-794 98 Orsa, SWEDEN

<sup>5</sup>Norwegian Institute for Nature Research, P.O. Box 5685 Torgard, NO-7485 Trondheim, NORWAY

Hibernation is an evolutionary adaptation to harsh conditions during the winter period. Finding a good place to hibernate is therefore crucial for survival and reproduction (e.g. RUF *et al.*, 2012). Brown bears (*Ursus arctos*) give birth during winter denning, and the type of den used for hibernation might affect the reproductive success of a female. Den type selection in brown bears varies by sex, age, and reproductive status (FRIEBE *et al.*, 2001; MANCHI and SWENSON, 2005). In Scandinavia, bears use four main types of dens, i.e., excavated anthills, above-ground nests, rock cavities and excavated soil dens (ELFSTRÖM and SWENSON, 2009). We investigated if den type affected female fitness, measured as a) the probability of giving birth to a litter, b) litter size, and c) the number of male cubs in a litter. Data from 109 females > 3 years old and 230 dens from 1987 - 2013 were used for analyses. We hypothesised that females in anthill dens would have a higher probability of giving birth (i.e., cub presence), produce larger litters, and have more male cubs than females using other den types. We used logistic generalized mixed effect models (logistic and Poisson) to test our hypotheses. We found that the age of the mother positively affected all three parameters. Den type had no effect on cub presence, but females hibernating in anthill dens had a significantly larger litter size. In addition, there was a trend for females giving birth in anthill dens to have more male cubs. Anthill dens are likely advantageous for hibernating bears in terms of energy conservation due to good insulation, independence of snow cover, and physical stability. Our findings suggest that anthill dens are important resources that positively affect reproduction rates in Scandinavian brown bears. Clear-cutting, today's most common forest harvest technique in Scandinavia, can be detrimental for mound building ants (SORVARI and HAKKARAINEN, 2005), and can thus have unexpected and indirect negative effects on sympatric species, such as brown bears. We suggest that forest management units should consider negative side effects of clear-cutting in their management to minimize damage on wildlife and biodiversity.

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## Assessing the conservation genetic value of zoo breeding programmes

OGDEN ROB<sup>1</sup>, ITO HIDEYUKI<sup>2</sup>, KING TONY<sup>3</sup>, SATO YU<sup>2</sup>, MURAYAMA MIHO<sup>2</sup>

<sup>1</sup>Royal (Dick) School of Veterinary Studies and The Roslin Institute, University of Edinburgh, Easter Bush Campus, EH25 9RG, Edinburgh, UK; rob.ogden@ed.ac.uk

<sup>2</sup>Wildlife Research Center of Kyoto University, Kyoto, 606-8203, JAPAN

<sup>3</sup>The Aspinall Foundation, Port Lympne Wild Animal Park, Lympne, Kent, CT21 4PD, UK

The application of genetic data to the management of captive breeding programmes for species conservation is increasing. A number of laboratory DNA methods are available to generate species, population and individual measures that can help inform managers about a range of issues from taxonomy to familial relatedness (FIENIEG and GALBUSERA, 2013). At a theoretical level, studbook management utilises assumptions about genetic diversity to evaluate breeding programme performance through common indices, and to infer the sustainability of populations in captivity. However to date, there have been few studies that investigate either; i) the genetic diversity of captive breeding programmes relative to natural populations in the wild, or ii), that aim to validate theoretical estimates of gene diversity in captivity against molecular genetic data. An understanding of these two issues is important to zoo population management if we are to seriously consider how captive populations may contribute to species conservation. This paper presents a number of recent examples from zebra, antelope and eagles where molecular genetic data from nuclear and mitochondrial DNA markers are applied to assess the conservation genetic value of zoo breeding programmes.

In zebra, a comparative analysis of genetic diversity using molecular and studbook estimates in Grevy's and Hartmann's Mountain zebra reveals counter-intuitive patterns of genetic variation, given the underlying theoretical performance of the two programmes (ITO *et al.*, 2016). For antelope, the distribution of wild lineages within managed herds informs the long-term utility of captive populations in several species; while in the Japanese golden eagle, a molecular study of wild and newly established captive conservation populations is combined with population viability analysis to plan the long-term genetic sustainability of the zoo breeding programme.

Taken together, these case studies highlight the need to challenge existing assumptions surrounding conservation breeding and to increase the level of baseline genetic data for the populations we may rely on for future reintroductions.

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## High-resolution measurement of social interactions of free-ranging dogs in rural Chad using proximity sensors

OZELLA LAURA<sup>1</sup>, WILSON-AGGARWAL JARED<sup>2</sup>, TIZZONI MICHELE<sup>1</sup>, CATTUTO CIRO<sup>1</sup>, McDONALD ROBBIE<sup>2</sup>

<sup>1</sup>ISI Foundation, Via Chisola 5, 10126, Turin, ITALY; laura.ozella@gmail.com; laura.ozella@isi.it

<sup>2</sup>University of Exeter, Environment and Sustainability Institute, Cornwall TR10 9FE, UK

Measuring social interactions between animals is a key challenge in the field of behavioural ecology. The large amount of data collected by proximity loggers can be used to develop quantitative contact networks, providing novel insights into the dynamics of social processes.

We used proximity loggers to detect close-range interactions between free-roaming dogs (*Canis lupus familiaris*) living in four villages in rural Chad for up to 14 days of deployment. The devices are lightweight (< 6 g, battery included), inexpensive, energy-efficient, and allow automated acquisition of a large amount of data (about 5 M of contact events for each sensor) with high temporal resolution. The study included 138 dogs, 60 females and 78 males, classified by age as: pups (< 6 months), juveniles (6 months – 1 year), sub-adults (1 – 2 years), and adults (> 2 years). Every dog belonged to one of 91 different households, with 32 households hosting more than one dog.

We generated aggregated contact networks, and we computed the number of contact events and the time spent in proximity for each pair of dogs. Contacts occurred mostly between dogs living in the same household, however, 98 % of the dogs had contacts with dogs living in a different household. Results showed that the pups are more sociable with dogs of the same litter, while dogs more than 6 months old tend to interact with dogs belonging to different households. With respect to their gender, contacts occurred mainly between males and females both within and across households. The temporal evolution of the number of contacts showed some distinct temporal features, specifically daily oscillations with two activity peaks: in the morning (6 AM – 8 AM) and in the evening (6 PM – 8 PM).

Our study shows the feasibility of accurate measures of contact patterns among free-roaming dogs in a rural context in Africa through the use of proximity sensors. Future applications of this system are expected to be useful for tracking many species, thus enhancing our understanding of the ecology and behaviour of free-living animals, as well as of their epidemiological consequences.

## **Non-invasive physiological stress and reproductive patterns of free-living European wildcats**

PIÑEIRO ANA<sup>1,2</sup>, BARJA ISABEL<sup>1</sup>, SILVÁN GEMA<sup>3</sup>, ILLERA JUAN CARLOS<sup>3</sup>

<sup>1</sup>Departamento de Biología, Unidad de Zoología, Facultad de Ciencias. Universidad Autónoma de Madrid. Ciudad Universitaria de Cantoblanco, C/ Darwin, 2. P.C: 28049 Madrid, SPAIN; ana.pineiro@unab.cl

<sup>2</sup>Escuela Medicina Veterinaria, Facultad Ecología y Recursos Naturales, Universidad Andrés Bello. Av. República 440, P.C: 8320000. Santiago de Chile, CHILE

<sup>3</sup>Departamento de Fisiología, Facultad de Veterinaria, Universidad Complutense de Madrid, SPAIN

The European wildcat (*Felis silvestris*) is a solitary and nocturnal species, therefore difficult to study under natural conditions. For this reason, the reproductive biology of the free-living wildcats is one of the least known aspects. The use of non-invasive methodologies such as the analysis of hormones in faeces, allows us to know different aspects of their ecology and behaviour. The present study was carried out in a protected area in Spain and the objectives were: 1) to analyse seasonal variations in sex hormone concentrations and 2) to evaluate variations in the levels of physiological stress in relation to the reproductive period. Hormonal analyses were performed on fresh faecal samples, which were collected in transects established by forest roads in the study area, through 4 years of study. Quantification of steroid hormone concentrations was performed by a competition immunoassay enzyme using the ELISA technique. The results showed significant differences in sex hormone concentrations throughout the year, with higher progesterone and estradiol levels in spring, followed by summer, winter and fall. However, testosterone levels were significant higher in summer, followed by spring, winter and fall. Finally, faecal cortisol metabolite (FCM) levels showed also a seasonal pattern: the highest concentrations in spring, followed by autumn, summer and winter. The results of the generalized linear model indicated that sex hormone levels and the season were the factors that explained the variation in FCM levels. The results indicate that the wildcat shows a seasonal reproductive pattern and that during this time the levels of physiological stress increase in the individuals. The results of this study are a useful tool to establish more effective conservation strategies for the species, since subjecting the species to stressors during a critical period such as the reproductive period can contribute to the decline of their populations in certain species.

## What affects intraspecific genetic structure in planktivorous seabird species?

PSHENICHNIKOVA OLESYA<sup>1</sup>, KLENOVA ANNA<sup>2</sup>, SOROKIN PAVEL<sup>3</sup>, KHARITONOV SERGEY<sup>3</sup>, ZUBAKIN VICTOR<sup>3</sup>, ARTUKHIN YURI<sup>4</sup>, SCHACTER CARLEY<sup>5</sup>

<sup>1</sup>National Research Center for Hematology of the Ministry of Health of the Russian Federation, Laboratory of Genetic Engineering, Novy Zykovsky pr. 4a, 125167, Moscow, RUSSIA; pshenichnikovaolesya@gmail.com

<sup>2</sup>Lomonosov Moscow State University, Dept. of Biology, Vorobiovi Gori 1/12, 119899, Moscow, RUSSIA

<sup>3</sup>AN Severtsov Institute of Ecology and Evolution RAS, Leninskiy pr. 33, 119071, Moscow, RUSSIA,

<sup>4</sup>Kamchatka Branch of Pacific Geographical Institute of Far Eastern Branch RAS, Rybakov pr. 19a, 683024, Petropavlovsk-Kamchatsky, RUSSIA

<sup>5</sup>Memorial University of Newfoundland, St. John's, NL A1C 5S7, Newfoundland and Labrador, CANADA

The comparison of intraspecific structure and biology in closely related species may allow us to reveal the factors influencing population differentiation. In this study we analysed intraspecific structure based on the mitochondrial control region in four planktivorous alcid species breeding in the North Pacific – crested auklet (*Aethia cristatella*, CA), whiskered auklet (*A. pygmaea*, WA), parakeet auklet (*A. psittacula*, PA) and ancient murrelet (*Synthliboramphus antiquus*, AM). We then compared the biology of these species and discussed possible factors affecting the formation of population structure. We collected 40 - 128 samples of each species from 4 - 5 sites and amplified the fragment of the control region (408 - 670 bp) with primers CGL-001, CGH-549, and additionally ca\_cr652L, ca\_cr960H for WA. We found that haplotypic diversity ( $h$ ) was relatively high in all species studied:  $h(\text{CA}) = 0.99 \pm 0.001$ ,  $h(\text{WA}) = 0.99 \pm 0.006$ ,  $h(\text{PA}) = 0.99 \pm 0.006$ ,  $h(\text{AM}) = 0.94 \pm 0.017$ . Nucleotide diversity ( $\pi$ ) was relatively high in three species:  $\pi(\text{CA}) = 0.014 \pm 0.008$ ,  $\pi(\text{PA}) = 0.012 \pm 0.007$ ,  $\pi(\text{AM}) = 0.01 \pm 0.005$ , but in WA it was half the size ( $\pi = 0.006 \pm 0.004$ ). We found no intraspecific structure in CA or AM ( $\Phi_{\text{ST}}(\text{CA}) = 0.018$  and  $\Phi_{\text{ST}}(\text{AM}) = 0.005$ ,  $p > 0.05$ ). In WA and PA we detected differentiation between American and Asian groups ( $\Phi_{\text{ST}}(\text{WA}) = 0.095$ ,  $\Phi_{\text{ST}}(\text{PA}) = 0.084$ ,  $p < 0.05$ ). Coalescent analysis (in IMA2) showed that mitochondrial lineages of these groups diverged about 12.6000 years ago in WA and about 92.000 years ago in PA. In WA, the Asian group consisted of two subgroups: Commander Islands and islands of the Okhotsk Sea ( $\Phi_{\text{ST}} = 0.074$ ,  $p < 0.05$ ), which diverged about 36.000 years ago. Our results indicated that intraspecific structure in WA and PA was shaped in the late Pleistocene as a result of range fragmentation into two refuges. The same did not occur in their relatives, CA and AM, probably because they form colonies with higher density and larger numbers of birds than WA and PA, and so need much more time to shape genetic differentiation. Also, WA and PA feed much closer to colonies than CA and AM, and migration behaviour differs among these species. At least in the Bering Sea WA and PA tend to winter within the sea where they breed, while CA and AM travel considerably within their range after the breeding season. Thus, the main factors influencing intraspecific genetic structure for seabirds with similar range, diet and ocean regime are probably factors that determine species mobility, and, particularly, migration behaviour, distance to feeding grounds and natal fidelity.

## **Artificial light at night, sleep debt and ecophysiology in wild developing songbirds**

RAAP THOMAS<sup>1</sup>, PINXTEN RIANNE<sup>1,2</sup>, EENS MARCEL<sup>1</sup>

<sup>1</sup>University of Antwerp, Department of Biology, Behavioural Ecology and Ecophysiology Group, Universiteitsplein 1, 2610, Wilrijk, BELGIUM; thomas.raap@uantwerp.be

<sup>2</sup>University of Antwerp, Faculty of Social Sciences, Antwerp School of Education, Venusstraat 35, 2000, Antwerp, BELGIUM

Artificial light at night (ALAN) is a widespread and increasing environmental pollutant with known negative impacts on animal physiology and development. Physiological effects could occur through sleep disruption and deprivation, but this is difficult to quantify, especially in small developing birds. Sleep loss can potentially be quantified by using oxalate, a biomarker for sleep debt in humans and rats. We examined the effect of ALAN on oxalate in free-living developing great tits (*Parus major*). Nestlings' physiology was quantified at baseline (= 13 days after hatching) and again after a two night exposure to ALAN. Subsequently, changes in oxalate were related to effects of ALAN on several parameters of physiological condition in the same individuals: haptoglobin, nitric oxide and body mass.

We found that ALAN increased oxalate levels but only in male nestlings, rather than decreasing it as was found in sleep deprived humans and rats. However, these changes in oxalate were not related to any change in physiological parameters. If oxalate is a reliable marker of sleep loss in developing great tits, our results suggest that effects of ALAN on physiology are not driven by disrupted sleep. Deterioration of nestlings' physiological condition is likely directly caused by ALAN or through other behavioural changes, like increased nestling activity.

## **Constraints on nocturnal activity mediate climate impacts on a tropical carnivore**

RABAIOTTI DANIELLA<sup>1,2</sup>

<sup>1</sup>Institute of Zoology, Zoological Society of London, Regents Park, NW1 4RY; UK

<sup>2</sup>Center for Biodiversity and Environment Research, University College London, Gower Street, London, WC1E 6BT; UK

Climate change is widely accepted to be one of the greatest threats to species globally. Identifying the species most at risk is therefore a conservation priority. Previous vulnerability assessments have highlighted ectothermic species at greatest risk of climate change as direct temperature impacts on these species have been identified. Recent findings, however, suggest that the African wild dog – a tropical mammal – may be impacted by rising temperatures, with consistently lower reproductive success when pup-rearing coincides with periods of high ambient temperature. Mammals exhibit complex behaviours, and can remain active across a wider range of temperatures than most endotherms, meaning that behavioural changes may have the potential to buffer the impacts of rising temperatures. Here we assess wild dogs' potential to adapt to high temperatures by switching to nocturnal hunting. Our findings have implications for the future conservation of the African wild dog, and highlight the need for behaviour to be considered within approaches to assessing climate change risk.

## **Trait responses of terrestrial animals to global warming are not adaptive**

RADCHUK VIKTORIIA, COURTIOL ALEXANDRE, KRAMER-SCHADT STEPHANIE

Leibniz Institute for Zoo and Wildlife Research (IZW), Dept. of Evolutionary Ecology, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; radchuk@izw-berlin.de

Climate change detrimentally affects species, with negative implications for biodiversity and associated with it ecosystem functioning. Therefore, understanding how species can adapt to climate change is crucial for the development of efficient conservation measures. We performed a literature review of 10090 abstracts and assembled data from 59 relevant publications into the ‘PACT’ dataset (‘Phenotypic Adaptation to Climate’). We then applied a meta-analysis to the PACT dataset to assess how terrestrial animals adapt to climate change. We showed that global warming has not affected morphological but advanced phenological traits of birds, the most represented taxa in our dataset. Advancement of phenology, however, was not adaptive as evidenced by the absence of a significant across-year selection in the direction conferring fitness benefits. We encourage further efforts directed to both contributing records to the PACT dataset in the future and using it to address adaptation-related questions across species and locations, which should serve as a basis for informed conservation measures.

## Dead or alive: most wanted mammals of Germany... elks, horseshoe bats and kin

RDUCH VERA, RULIK BJÖRN, HUTTERER RAINER, DECHER JAN & GEIGER MATTHIAS

Zoologisches Forschungsmuseum Alexander Koenig, Leibniz-Institut für Biodiversität der Tiere, Adenauerallee 160, D-53113 Bonn, GERMANY; v.rduch@leibniz-zfmk.de

Maintenance of high levels of biodiversity through conservation depends on correctly identified species. The GBOL (German Barcode of Life) project, funded by the German Federal Ministry of Education and Research, is a national DNA barcoding campaign for assessing the genetic diversity of animals, fungi and plants in Germany. The consortium of natural history collections, botanical gardens, and universities coordinates collecting, processing, data sharing and deposition of samples in conventional and molecular collections in order to facilitate the compilation of an open access DNA barcode reference database for the country's fauna and flora (GEIGER *et al.*, 2016). Professional taxonomists closely work together with enthusiastic and active external taxonomic experts, including committed citizen scientists, to establish this library of biodiversity.

In case of mammals the target list follows the German Red List (MEINIG *et al.*, 2009) and comprises 103 taxa at the species level. Focussing on terrestrial species it also includes vagrant species, as well as species that are extinct on a global or local scale. Until now, GBOL archived more than 1,200 samples, covering 87 % of the target taxa. Samples consist of tissue, blood or hair from classical museum specimens, dead or living animals.

The importance of the target list grows with the progress in taxonomic coverage achieved, as gaps can be identified and addressed more specifically. Since our goal is to cover the molecular diversity over the range of each species in Germany, we want to include several (2 - 7) specimens from each of the federal states. This pragmatic approach enables quick queries for the presence of taxa or the coverage of particular taxa and helps coordinating sampling efforts. Registered experts can use the web portal (<https://www.bolgermany.de/ergebnisse/results>) to retrieve the coverage of a particular taxon for each federal state.

Because there are still 'missing taxa' of mammals on the GBOL target list (<http://tinyurl.com/GBOL-mammal>) –also in other animal groups – we are looking for people from different fields that want to contribute by providing samples. Any inquiries and contributions that help increasing the number of taxa covered and improve the significance of the barcode reference library are highly welcomed!

GEIGER MF, ASTRIN JJ, BORSCH U, BURKHARDT U, GROBE P, HAND R, HAUSMANN A, HOHBERG K, KROGMANN L, LUTZ M, MONJE C, MISOF B, MORINIÈRE J, MÜLLER K, PIETSCH S, QUANDT D, RULIK B, SCHOLLER M, TRAUNSPUNGER W, HASZPRUNAR G, WÄGELE W (2016): How to tackle the molecular species inventory for an industrialized nation – lessons from the first phase of the German Barcode of Life initiative GBOL (2012-2015). *Genome* **59**, 661 - 670.

MEINIG H, BOYE P & HUTTERER R (2009): Rote Liste und Gesamtartenliste der Säugetiere (Mammalia) Deutschlands. – In: HAUPT H, LUGWIG G, GRUTTKE H, BINOT-HAFKE M, OTTO C, PAULY A (Red.): Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands. Band 1: Wirbeltiere. – Naturschutz und Biologische Vielfalt **70**, 115 - 153.

## Breeding and life history patterns of saiga antelopes (*Saiga tatarica*) at Cologne Zoo, Germany

RDUCH VERA<sup>1,2</sup>, SLIWA ALEXANDER<sup>1</sup>

<sup>1</sup>Zoologischer Garten Köln, Riehler Str. 173, 50735 Köln, GERMANY; sliwa@koelnerzoo.de

<sup>2</sup>Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, GERMANY; v.r duch@leibniz-zfmk.de

Cologne Zoo, Germany, was one of the most successful zoos holding and breeding saiga antelopes (*Saiga tatarica*) between 1976 and 2009 (RDUCH *et al.*, 2016). So far, zoos have failed in establishing a stable captive population of this critically endangered ungulate. We analysed precise breeding and life history data of individually known saigas from Cologne Zoo. By this we provide interesting data for zoo and field biologists, valuable if future captive breeding is intended and underline the potential of research in zoos. Rut happened during winter as in the wild. Oestrus length usually lasted 1 – 2 days ( $N_{\text{days\_with\_females\_in\_oestrous}} = 107$ ,  $N_{\text{females}} = 14$ ). If not gravid, females came in oestrus again after a mean period of 19.9 days ( $N_{\text{events}} = 20$ ,  $N_{\text{females}} = 7$ ). Gestations lasted 133 - 151 days. We found significant differences between gestation lengths for singletons (mean 142.8 days) and twins (mean 138.4 days) ( $U$ -Test:  $N = -2.010$ ,  $p = 0.044$ ,  $N = 18$ ). Births happened from April to June. Twins made up 78.0 % of the offspring of females born in Cologne ( $N_{\text{births}} = 35$ ) and only 20.7 % of the offspring of females that were brought to Cologne ( $N_{\text{births}} = 26$ ). Mean litter size and thus offspring per year was 1.3 or 1.4 young (henceforth the latter value concerns only the Cologne-born females), thus lower than reported from the wild where twin-birth are considered the rule. For one female maximum offspring number was eight born in four twin births. Singleton lambs were born to females of all age classes and twinning occurred in primiparous females. Considering only Cologne-born females, we only found low correlations between litter size and the female's age (Pearson Correlation:  $r = 0.343$ ,  $p = 0.043$ ,  $N = 35$ ) or its number of births (Pearson Correlation:  $r = 0.354$ ,  $p = 0.037$ ,  $N = 35$ ), respectively. Crucial for breeding is the life expectancy of females that grew significantly older than males ( $U$ -Test:  $Z = -2.624$ ,  $p = 0.009$ ,  $N = 75$ ). Females in Cologne were fertile from their first winter, like in the wild, and on average they lived 4.3 years ( $N = 35$ ). We found a discrepancy between the theoretical number (5.2 to 5.7) and the actual mean number of offspring (both 2.8) per female during their lives. This might be explained by constrains in the husbandry, among others by the temporary absence of a breeding male, or the suggested inability of some designated males to sire under the age of 2 years. We emphasise the need to safeguard continuous breeding, to prevent the loss of any of the few years in a female's breeding life.

RDUCH V, ZIMMERMANN W, VOGEL KH, LADENER H, SLIWA A (2016): Saiga Antilopen (*Saiga tatarica*) im Kölner Zoo: tiergärtnerische Erfahrungen und Beobachtungen. Zeitschrift des Kölner Zoo **59**, 135 - 149. (English version available)

## Measuring animal welfare: behaviour and physiology in zoo fossa (*Cryptoprocta ferox*)

REGAIOLLI BARBARA<sup>1</sup>, CALAMANDREI SILVIA<sup>2</sup>, ACCORSI PIER ATTILIO<sup>2</sup>, SPIEZIO CATERINA<sup>1</sup>

<sup>1</sup>Parco Natura Viva-Garda Zoological Park, Loc. Figara, 40, 37012, Verona, ITALY; barbara.regaiolli@parconaturaviva.it

<sup>2</sup>Facoltà di Agraria e Medicina Veterinaria, Università di Bologna, Viale Giuseppe Fanin, 40127 Bologna, ITALY

Animal welfare is a state of both physical and mental health of an animal, which is capable of coping with the environment and perform a wide array of species-specific behaviours (HILL and BROOM, 2009; HOSEY *et al.*, 2013). To assess animal welfare, behavioural and physiological indicators can be used (SEJAN *et al.*, 2011). Fossa (*Cryptoprocta ferox*) is a solitary carnivore species, therefore difficult to breed in captivity. This study aims at evaluating the welfare of a male and a female zoo fossa housed in two adjacent enclosures, through ethological and physiological parameters. Moreover, behavioural patterns displayed by the female and male fossa were used to assess whether they were ready for breeding. The study was made of five periods, in which subjects could or could not perceive each other directly from the enclosure mesh. Per period, twenty-four 30-minute sessions per subjects were done. A continuous focal animal sampling method was used to collect durations of normal and abnormal behaviours. Faecal samples were collected in both fossa to analyse the amount of cortisol, progesterone and testosterone. Results underlined that both subjects performed species-specific behaviours such as exploration and scent-marking. Abnormal behaviour was also reported and was performed more by the female than the male. Particularly, the proximity of the two subjects with each other affected the performance of abnormal behaviour, especially in the male. No relationship between the performance of abnormal behaviour and the cortisol level was reported. Finding from this study provided useful information on the behavioural biology of zoo fossa, helping to make informed decision on the socialization process between the study subjects.

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HOSEY G, MELFI V, PANKHURST S (2013): *Zoo Animals: Behaviour, Management and Welfare*, Oxford University Press.

SEJANV, LAKRITZ J, EZEJI T, LAL R. (2011): Assessment methods and indicators of animal welfare. *Asian J Anim Vet Adv* **6**, 301-315.

## Rise of a lynx population in Central Europe - Genetic evaluation of reintroduction success after 15 years

REINERS TOBIAS ERIK<sup>1</sup>, MIDDELHOFF LILLI<sup>2</sup>, ANDERS OLE<sup>2</sup>, NOWAK CARSTEN<sup>1</sup>

<sup>1</sup>Senckenberg Research Institute and Natural History Museum Frankfurt, Conservation Genetics Group, Germany, Clamecystrasse 12, 63571, Gelnhausen, GERMANY; tobias.reiners@senckenberg.de

<sup>2</sup>Nationalparkverwaltung Harz, Luchsprojekt Harz, Außenstelle Oderhaus, 37444, Sankt Andreasberg, GERMANY;

Large carnivores, such as wolves and lynx, are expanding their ranges across Central and Western Europe. In contrast to wolves, Eurasian lynx (*Lynx lynx*) were actively reintroduced in the frame of several projects. A comparably recent reintroduction was launched in the Harz Mountains National Park, Central Germany. Between 2000 and 2006, 24 lynx of different origins were released (nine males, 15 females). In this study, we describe the spread and genetic properties of this reintroduced lynx population since the beginning of the project.

To quantify the spatial spread, we compiled lynx observations since 2000, using the SCALP framework and summarized these using the 10 km × 10 km map grids (EEA reference Grid). For genetic analysis, we used 379 samples, collected in the wild between 2001 and 2016, including 41 tissue samples, 66 blood samples, and 272 non-invasive samples (118 hair samples, 45 scats and 109 saliva samples). Samples were analysed for two mtDNA loci. Confirmed lynx samples were genotyped at 19 microsatellite loci and two sex markers using a multiple tubes approach. Genotype quality was assessed by amplification success and genotyping errors. Analysis of population ancestry of sampled individuals was performed with STRUCTURE, including lynx from the Bohemian-Bavarian population and individuals from wildlife parks. After confirming population ancestry, parent-offspring associations were tested per monitoring year. Combining individual life history data from catches, telemetry, and photo trapping with genetic sampling enabled us to include additional information into parentage inference using COLONY, thereby avoiding arbitrary complexity. The derived pedigree was used to calculate inbreeding and kinship coefficients and to estimate the number of generations. SCALP data on the Harz population showed that the number of 10 km × 10 km squares occupied tripled since 2009/10. STRUCTURE analysis revealed that 106 wild born individuals from the Harz region form a defined genetic cluster different from founders, captive lynx and those from the Bohemian-Bavarian population. The large number of genotyped individuals and information from the field was used to reconstruct a substantial part of the wild pedigree starting from reintroduced individuals in 2001 to animals born in 2016, spanning already five generations. The pedigree indicates a slight increase in inbreeding, whereas the genetic data revealed a relatively high genetic diversity compared to founder individuals and other reintroduced lynx populations.

## **Circadian variations in faecal androgen metabolites in captive red brocket deer (*Mazama americana*)**

ROLDAN MAR, GALINDO DAVID, SANDOVAL ELUZAI, TANAKA YUKI, DUARTE J. MAURÍCIO

Deer Research and Conservation Center (NUPECCE), São Paulo State University, Faculty of Agriculture and Veterinary Sciences, Department of Animal Sciences, Via de Acesso Professor Paulo Donato Castellane s/n, Jaboticabal, SP, 14884-900, BRAZIL; roldan.romero.m@gmail.com

Studies about hormone profiles in deer and their effects on deer biology are very useful to determining physiological conditions such as reproductive status and stress of individuals. Gonadal status of animals can be assessed accurately through blood samples, but deer species are characterized by a highly stressful response to handling, therefore non-invasive methods are more appropriate to monitor hormone concentrations in these species. Faecal collection has proven to be a non-invasive method of obtaining samples that can be analysed for androgen metabolites in deer. Despite *Mazama americana* is fairly common and widespread in forested areas, this species is classified as DD (Data Deficient) by the IUCN Red List. Red brocket deer exhibit nocturnal activity patterns and have on average approximately 19 hours of gut-passage time. This is relevant when planning experiments and collecting faecal samples of this species. A previous study showed that *M. americana* does not exhibit an annual pattern in androgen secretion, and to date, no studies exist that measured daily faecal androgens metabolites (FAM) concentration in any *Mazama* species. Therefore, this work provides a better understanding about circadian secretion of hormones in neotropical deer species. This study was performed using 103 faecal samples collected throughout the day from five individually housed males, every three hours for a period of three days. The FAM concentration in samples were measured with a competitive testosterone enzyme immunoassay (EIA) (R156/7; provided by C. Munro). FAM concentrations showed a slight tendency to increase from the last hours of the evening to the early morning hours, however, there was no relationship between the collection time and FAM concentrations throughout a day ( $p > 0.05$ ). These findings might indicate that red brocket deer have no circadian pattern in FAM secretion, suggesting that testicular function in this species is little or not affected by a circadian stimulus.

Acknowledgements: 2016/12521-3, São Paulo Research Foundation (FAPESP)

## **A picture is worth a thousand words – increasing scientific literacy by conveying scientific results through images**

ROELLIG KATHLEEN, WEYRICH ALEXANDRA, BRANDT MIRIAM

Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; roellig@izw-berlin.de

The Leibniz Institute for Zoo and Wildlife Research (IZW), in accordance with its mission „Evolutionary wildlife research for conservation“, investigates the many adaptations developed by wildlife in the evolutionary process and develops novel concepts and methods to aid the conservation of threatened wildlife species.

In two pilot projects, we “translated” scientific publications (LEHMANN *et al.*, 2013; WEYRICH *et al.*, 2015) into (1) a children’s book designed for pre-school and primary school children (ROELLIG and GUMPERT, 2016) and (2) a science comic aimed at students in senior classes and adults (WEYRICH *et al.*, 2016).

“The Magic Trick” (1) presents the scientific message how herbivores can survive in extremely scarcely vegetated deserts. The story entwines around the three friends Bono the hare, Jinny the mouse and Otto the bat living under the hot African sun. One morning, a stranger comes to the desert. The three friends follow him and find they have embarked on quite an adventure.

In “Epigenetics – Bridge between genome and environment” the reader will learn about how changes induced by environmental factors can be transmitted to the next generation. Just like a piano keyboard, the genome in each cell of a living being is identical, while epigenetics determines the song that is played. In this comic, Ada tells us how epigenetics composes these songs.

Through this form of representation, we aim to increase knowledge about the mechanisms of adaptation and evolution, and foster an understanding of the research process and scientific methodologies applied. To reach a broad audience both books are available in German and English; “The Magic Trick” also in Afrikaans and Damara to distribute it to local schools in Namibia.

LEHMANN D, MFUNE JKE, GEWERS E, CLOETE J, BRAIN C, VOIGT CC (2013): Dietary Plasticity of Generalist and Specialist Ungulates in the Namibian Desert: A Stable Isotopes Approach. PLoS ONE **8**: e72190.

WEYRICH A, LENZ D, JESCHEK M, CHUNG TH, RÜBENSAM K, GÖRITZ K, JEWGENOW, K, FICKEL J (2016): Paternal intergenerational epigenetic response to heat exposure in male Wild guinea pigs. Mol Ecol **25**, 1729-40.

RÖLLIG K, GUMPERT S (2016): Bonos Abenteuer – Der Supertrick / Bono’s adventure – The Magic Trick.. IZW, Berlin.

WEYRICH A, NOWACKI O, KÖHN A (2016): Epigenetik – Brücke zwischen Erbgut und Umwelt. Ein Wissenschafts-Comic des Leibniz-Instituts für Zoo- und Wildtierforschung Berlin (IZW). / Epigenetics – Bridge between genome and environment. Science Comic - Leibniz Institute for Zoo and Wildlife Research (IZW). Jaja-Verlag, Berlin.

## Restoration of the North Persian leopard in the Russian Caucasus: the experience of pre-releasing assessment and the first results of released animals monitoring

ROZHNOV VIATCHESLAV V<sup>1</sup>, YACHMENNIKOVA ANNA A<sup>1</sup>, CHISTOPOLOVA MARIA D<sup>1</sup>, TREPET SERGEY A<sup>2</sup>, PKHITIKOV ALIM B<sup>2</sup>, SOROKIN PAVEL A<sup>1</sup>, NAIDENKO SERGEY V<sup>1</sup>, DRONOVA NATALIA A<sup>3</sup>, HERNANDEZ-BLANCO JOSE A<sup>1</sup>

<sup>1</sup>A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Science, Leninsky pr. 33, 119071, Moscow, RUSSIA; felis.melanes@gmail.com

<sup>2</sup>A.K. Tembotov Institute of Mountainous Area Ecology, Russian Academy of Sciences, Armand str., 37, Nalchik, 360051, RUSSIA

<sup>3</sup>WWF, Nikoloyamskaya str., 19/3, Moscow, 109240, RUSSIA

The project for restoration of the North Persian leopard (*Panthera pardus saxicolor*) in the Russian Caucasus began at 2007; it was supported by IUCN and EAZA. A specialized centre for leopards breeding and rehabilitating was built. Two unrelated breed pairs were established. The kittens they produced were prepared for released into the wild when adult. In July 2016 on the territory of the Caucasian Biosphere Nature Reserve (MAB UNESCO) the first captive bred leopards were released. A system for assessing the animal readiness for their release into the wild was developed and implemented. It includes an assessment of daily behaviour of animals and five experimental tests. Tests include an assessment of the ability of animals to hunt (a positive stimulus); reaction to human (negative stimulus); evaluation of the reaction to livestock (negative stimulus in combination with positive hyperstimulation). As a maximum, each animal can score 70 points and be rated as "fit for release into the wild". We tested four leopards. Three of them were recognized as fit enough for releasing - one female (three years old) and two males (two and three years old). Three released leopards were fitted with GPS-Iridium collars (LOTEK) that was equipped with a VHF transmitter. Transmitters provided a fix for the animal's location every two hour; monitoring specialists obtain information once a day. Home range estimation was built by two methods (MCP 100 % and kernel 95 %). The criterion to estimate the completeness of a leopard's home range was the degree of changes noted in the calculated area used by a leopard over time (i.e. when this tended towards the asymptote). Clusters of consecutive locations at the same place during 24 hours were assumed to be kill sites and inspected *in situ* in order to estimate leopards hunting success. We installed photo traps in the area where leopards were released. To April 2017: one male has his collar removed; one male continued to expand its range; the home range of the female is completely formed. The prey species killed by the released leopards included wild boars (*Sus scrofa*), tours (*Capra caucasica*), chamois (*Rupicapra rupicapra*), deer (*Cervus elaphus*) and wolves (*Canis lupus*). Female has a more diverse diet than males; uses the kill more fully, staying there longer ( $72 \pm 48$  h), hides killed prey better. Males spend less time period near the kill ( $61.0 \pm 43.0$  h and  $67 \pm 46$  h); often lose killed prey because of competitors. Given the current small sample sizes difference in behaviour between males and females is conjecture.

## Behavioural genetics of personality traits in the Eurasian harvest mouse (*Micromys minutus*)

RUIZ FADEL FERNANDA, SCHUSTER ANDREA, THOMASSEN HENRI, FOERSTER KATHARINA

University of Tübingen, Institute of Evolution and Ecology, Comparative Zoology, Auf der Morgenstelle 28, D-72076, Tübingen, GERMANY; ferufa@gmail.com

The study of personality traits aims to understand variation, fitness, and evolutionary benefits in animals. In recent years, the study of animal behaviour has been combined with quantitative genetics, exploiting the advances in next generation sequencing. These developments allow for quantifying associations between behavioural traits and genes. Animal personality has been described as the constant between-individual behavioural differences throughout time and across situations. Previous work using Eurasian harvest mice (*Micromys minutus*) has shown that the personality traits “boldness”, “activity” and “exploration” are repeatable within life history stages and consistent between life history stages in this species (SCHUSTER *et al.*, 2017a; SCHUSTER *et al.*, 2017b). Repeatability and consistency of the phenotypic traits and correlations between them suggest a genetic basis, therefore making them good candidate traits for studying associations between behaviour and underlying genetic variation. Here, we describe the workflow to look for associations between single nucleotide polymorphism (SNPs) and the traits “boldness”, “activity” and “exploration” in harvest mice using next generation sequencing (NGS). We also report preliminary association results for these traits based on measurements from previous projects of a laboratory population and a semi-natural population of Eurasian harvest mice.

SCHUSTER AC, CARL T, FOERSTER K (2017a): Repeatability and consistency of individual behavior in juvenile and adult Eurasian harvest mice. *The Science of Nature* **104**, 10.

SCHUSTER AC, ZIMMERMANN U, HAUER C, FOERSTER K (2017b): A behavioural syndrome, but less evidence for a relationship with cognitive traits in a spatial orientation context. *Front Zool* **14**, 19.

## Relationships between industrial metal exposure and oxidative status in an insectivorous bat

RUIZ SANDRA<sup>1</sup>, EEVA TAPIO<sup>1</sup>, KANERVA MIRELLA<sup>1</sup>, LILLEY THOMAS<sup>1,2</sup>

<sup>1</sup>University of Turku, Faculty of Mathematics and Natural Sciences, Department of Biology, FI-20014, Turku, FINLAND; srruiz@utu.fi

<sup>2</sup>University of Liverpool, Evolution, Ecology and Behaviour, Institute of Integrative Biology, Crown Street, L69 7ZB, Liverpool, UK

Exposure to heavy metal elements from industrial sources can have negative consequences on ecosystems. Particularly, long-lived animals may be vulnerable to the effects of low but prolonged metal exposure because accumulation of toxicants interferes with important physiological functions e.g. mineral deposition in bones, micronutrient gastrointestinal absorption and nervous system development. Bats can accumulate metals in internal organs. However, scarce information exists about the effects of metal exposure on their physiological status. Oxidative status markers are known to be altered after detoxification processes and immune responses. Here, we studied individuals of an insectivorous bat, *Myotis daubentonii*, from natural populations inhabiting close to a metal emission source and a less contaminated site for two consecutive summers. We quantify metal elements (As, Ca, Cd, Co, Cu, Mn, Pb, Ni, Se, Zn) from individual faecal pellets and measured antioxidant enzyme activities (GP, CAT, SOD, GSH:GSSG) from red blood cells. We also report biometrics, haematocrit and parasite prevalence. In general, metal concentrations in faeces of *M. daubentonii* reflected the exposure to ambient contamination. This was especially evident in the higher concentrations of Cd, Co, Cu and Ni close to a smelter compared to a place with less contaminant exposure. Year and site differences were also observed for most elements quantified. Calcium and zinc differed between males and females, while SOD and CAT correlated with principal components of six toxic metals, suggesting an effect of metal exposure on enzymatic activities when metals occurred at elevated concentrations.

## **Analysis of vocal rutting activity across populations of red deer (*Cervus elaphus*): effects of rut phase, time of day and ambient temperature**

RUSIN IVAN Y<sup>1</sup>, VOLODIN ILYA A<sup>1,2</sup>, VOLODINA ELENA V<sup>2</sup>

<sup>1</sup>Department of Vertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Vorobievsky Gory, 1/12, Moscow, 119234, RUSSIA, kikimos@yandex.ru

<sup>2</sup>Scientific Research Department, Moscow Zoo, B. Gruzinskaya str., 1, Moscow, 123242, RUSSIA

In polygynous red deer *Cervus elaphus*, a prolonged rutting vocal activity represents a prominent part of male reproductive behaviour. Duration of the rut as well as the overall vocal activity differ between populations and between years. This study compares stag vocal rutting activity across populations of red deer. Data were collected in September-October 2015 using 5 SongMeter 2+ automated recording devices, scheduled for 5 min/hour, 24 hours/day, 60 - 70 days per each device in 3 populations of red deer: Ussuri (*C.e. xanthopygus*, 2 devices), Lipetsk, (*C.e. hippelaphus*, 1 device), Kostroma (*C.e. sibiricus*, 2 devices). For each device, call dynamics had a humped shape with one maximum. We calculated daily mean call number/hour for each 24-hour period. We selected days when call number/hour reached 1 % of maximum as days of rut onset and completions. We calculated the mean call number/hour for the entire rut period and subdivided rut period into beginning phase (between onset day and day with mean call number/hour), active phase (between two days with mean call number/hour) and end phase (from day with mean call number/hour to the end day). Vocal activity was highest in Kostroma (maximum 331 and 351 calls/hour), intermediate in Lipetsk (271 calls/hour) and weakest in Ussuri (46 and 19 calls/hour). Rut duration was longest in Lipetsk (58 days), intermediate in Kostroma (52 days) and shortest in Ussuri (45 days). However, the active phase was longest in Kostroma (25 days), intermediate in Lipetsk (20 days), and shortest in Ussuri (12 days), probably in relation to differences in animal density, highest in Kostroma and lowest in Ussuri. Separately for each rut phase, we estimated effects of time of day and ambient temperature on call number/hour with GLMM. Time of day significantly affected call number/hour for the beginning phase in two cases (Lipetsk and Kostroma1), for the active phase in five cases (all devices) and for the end phase in two cases of the five (Kostroma1,2). Temperature significantly affected call number/hour for the beginning phase in one case (Ussuri1), for the active phase in three of the five cases (Ussuri, Kostroma1,2), and never for the end phase. Thus, temperature had lower effect on call number/hour compared to time of day. Data on more populations and examining effects of other weather factors are necessary for the conclusive data on effects of environment factors on vocal rutting activity across populations of red deer. Support: RFBR grant 15-04-06241.

## **Caucasian brown bear genetics reveals complicated structure and history of the population**

SALOMASHKINA VALENTINA<sup>1</sup>, KHOLODOVA MARINA<sup>2,3</sup>, SEMENOV UMAR<sup>4</sup>, MURADOV AZERCHIN<sup>5</sup>, MALKHASYAN ALEXANDER<sup>6</sup>

<sup>1</sup>National Research Center for Hematology of the Ministry of Health of the Russian Federation, Laboratory of genetic engineering, Novy Zykovsky pr. 4a, 125167, Moscow, RUSSIA; v-salomashk@yandex.ru

<sup>2</sup>A.N. Severtsov Institute of Ecology and Evolution RAS, Leninskiy pr. 33, 119071, Moscow, RUSSIA

<sup>3</sup>Tomsk State University, Lenin pr. 36, 634050, Tomsk, RUSSIA

<sup>4</sup>Sochi National Park, Moskovskaya str. 21, 354000, Sochi, RUSSIA

<sup>5</sup>Ilisu State Nature Reserve, Azerbaijan pr. 39a, 3400, Qakh, AZERBAIJAN

<sup>6</sup>WWF Armenia, Proshyan 19, 0019, Yerevan, ARMENIA

The brown bear population of the Caucasian region is among the least studied brown bear populations in the world. Caucasian bears have very diverse morphological traits and probably a complex origin. We performed a genetic analysis of 35 samples of brown bears from Caucasus and Transcaucasia based on a mtDNA control region fragment of 570 bp length and 10 autosomal microsatellite loci. Mitochondrial data showed four distinct haplogroups: one of them consisted of bears from the Northern slope of the Greater Caucasus, the second one included bears from both Northern and Southern slopes of the Greater Caucasus, the third one consisted of bears from the Southern Greater Caucasus only, and the fourth group consisted of bears from the Lesser Caucasus, Transcaucasia. The comparison of the obtained data with sequences obtained and deposited in GenBank by other researchers showed that those four haplogroups have different origins. Two Northern-Caucasian haplogroups were close to the Eastern-European clade, which is very homogenous and was found through Northern Eurasia from Eastern Europe to Chukotka and Kamchatka. None of these groups was directly included into the Eastern-European clade, and they did not form a sister relationship between each other either. The third group, consisting of the Southern-Greater Caucasus bears, formed a single haplogroup with bears from Southwestern Iran, while bears from the Lesser Caucasus belonged to the Northern Iranian clade. That makes a picture of wedge of the Northern Iranian clade, separating the Southern Iranian clade into two enclaves – one in the Southwestern Iran, another one at the Southern slope of the Greater Caucasus, with a few distinct clades, that were found at the North Caucasus and not related to any Iranian clade. Microsatellite analysis showed a clear differentiation between the Caucasian samples and samples from European Russia, but failed to reveal any population structure within the Caucasian sample. Taking into account the clear differentiation shown by the mtDNA data, this lack of structure can be considered as a result of male-biased dispersal behaviour.

Our data suggest that Caucasian brown bear population can be considered as a result of complex immigration and dispersal processes. This population includes descendants of a few immigration waves of different time periods and origins.

The study was supported by RBF grant 16-34-01083 mol\_a.

## **Applying conservation endocrinology to reptiles and frogs: possible pitfalls and shortcomings**

SCHEUN JUAN<sup>1</sup>, GANSWINDT ANDRE<sup>2</sup>

<sup>1</sup>National Zoological Gardens of South Africa, 232 Boom street, 0082, Arcadia, Pretoria, SOUTH AFRICA; juan@nzg.ac.za

<sup>2</sup>Endocrine Research Laboratory, Department of Anatomy and Physiology, University of Pretoria, Onderstepoort, 0110, Pretoria, SOUTH AFRICA

Conservation efforts for amphibian and reptile species are notorious for being plagued with difficulties in defining the stress status of individuals. Although endocrine monitoring offers a robust tool for monitoring the stress responses of a species, the sometimes peculiar biology and rather small body size of amphibians and reptiles can further limit the ability to conduct frequent sampling events as required. As such, the appropriate sampling matrix, sampling regime and enzyme immunoassays to be used for monitoring adrenocortical function need to be carefully validated for each species. At the National Zoological Gardens of South Africa we looked at the validity of using faecal samples in sungazers (*Smaug giganteus*) and mucus in the edible bullfrog (*Pyxicephalus edulis*) for monitoring respective hormone patterns. For the validation process of both *S. giganteus* and *P. edulis* we used biological stressors, such as handling and blood collection events, to activate the hypothalamic-pituitary-adrenal axis. Faecal samples of *S. giganteus* were collected for a month prior to and following the stressor. We used cotton swabs to collect frog mucus, for monitoring adrenal activity, following the handling event of *P. edulis*. Samples were extracted and analysed at the Endocrine Research Laboratory, University of Pretoria, South Africa. For *S. giganteus* we found no elevation in glucocorticoid metabolite concentrations following the stress event. Initial results for *P. edulis* indicated that mucus may be a reliable matrix for monitoring glucocorticoid alterations in amphibians. However, subsequent results did not confirm the initial findings. The results underline recent opinion that the suitability of an appropriate endocrine monitoring technique should not be exclusively verified via biological validation, but rather through the use of a physiological validation, such as an Adrenocorticotrophic Hormone Stimulation Test (ACTH Test). The validations may have failed for a number of reasons. Firstly, handling and blood collection practices may not successfully activate the hypothalamic-pituitary-adrenal axis. Secondly, sampling regimes may not have allowed for enough time to observe the increase in glucocorticoid production. Thirdly, hormone metabolism and excretion routes (via urine, faeces, mucus) differ distinctively within and between species. Mucus may well not be an ideal matrix for hormone monitoring in amphibians, though much more work is required before eliminating it as unviable. Similarly, the excretion of a combination of urine and faeces in *S. giganteus* may have resulted in indistinctive glucocorticoid metabolite concentrations. Further studies on amphibian and reptile species should consider these factors when evaluating methods concerning steroid metabolite quantification.

## **Stress in deer populations - possibly stronger effect of humans than predators**

SCHMIDT KRZYSZTOF<sup>1</sup>, ZBYRYT ADAM<sup>2</sup>, KUIJPER DRIES PJ<sup>1</sup>, BUBNICKI JAKUB W<sup>1</sup>, DEHNHARD MARTIN<sup>3</sup>

<sup>1</sup>Mammal Research Institute PAS, Waszkiewicza Str. 1, 17-230 Białowieża POLAND; kschmidt@ibs.bialowieza.pl

<sup>2</sup>The Polish Society for Bird Protection, Ciepła Str. 17, 15-471 Białystok, POLAND

<sup>3</sup>Leibniz-Institute for Zoo and Wildlife Research, Alfred Kowalke-Strasse 17, 10315 Berlin, GERMANY

We studied the stress level in red deer and roe deer in six populations exposed to potential stressors of different intensity including predation, hunting harvest, and several factors related to habitat structure and availability by analysing the faecal glucocorticoid metabolites (FGM). We predicted that predation may be a major factor in shaping the stress levels in ungulates, therefore we selected study areas mainly with regard to presence or absence of two large carnivores – the wolf and the lynx: two areas with native populations of both wolf and lynx, two areas recently recolonized by wolf and two areas with no large carnivores.

Considering averaged values per area we found highest FGM level in both roe deer and red deer in the areas where no large carnivores occurred, and lowest in areas with wolf and lynx. Therefore, the presence of predators could not be regarded as a major stressful factor to ungulates. Moreover, the FGM level in areas without predators was characterized by very high variance, whereas that in areas with both carnivores showed low variance. To explain what factors could influence observed FGM levels (both mean values and their variances) at the scale of single hunting district, we fitted a series of competing Double Hierarchical GLM (DHGLM) models, including: hunting pressure, density of build-up areas, forest cover, habitat fragmentation, undergrowth, ungulate density and the interactions among them, as explanatory variables. In case of red deer only hunting harvest significantly affected the variance of the FGM level. In roe deer, the percentage of built up areas in interaction with roe deer density most significantly contributed to elevating the average stress level and its variance. The results suggest that stress in ungulate populations is lower and less variable in areas utilized by large carnivores than in areas where human-related factors predominate. The mechanism of this phenomenon may be that prey animals show evolutionarily shaped behavioural and physiological adaptations to the risk associated with natural predators due to their constant exposure to it. In contrast, the risk resulting from human activities due to its exceptionally high intensity and high probability of risky encounters at individual level as well as its erratic occurrence disabled the animals' adaptive abilities, which is consistent with recent concept of humans as "super predators". This questions the role of hunting as a "substitute" of natural predation warranting analogous functioning of the ecosystem and should be carefully considered in wildlife management.

## **Does administration of antioxidants reduce oxidative stress and clinical signs of a viral disease in magnificent frigatebirds?**

SEBASTIANO MANRICO<sup>1</sup>, EENS MARCEL<sup>1</sup>, CHASTEL OLIVIER<sup>2</sup>, COSTANTINI DAVID<sup>3</sup>

<sup>1</sup>Behavioural Ecology and Ecophysiology group, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, BELGIUM; manrico.sebastiano@uantwerpen.be

<sup>2</sup>Centre d'Etudes Biologiques de Chizé (CEBC), UMR7372- CNRS/University of La Rochelle, F-79360, FRANCE

<sup>3</sup>UMR 7221, Muséum National d'Histoire Naturelle, 7 rue Cuvier 75231 Paris cedex 05, FRANCE

Although infectious diseases are widespread in animals, little is known about their effects on the physiological condition and survival. Some clinical studies have suggested that oxidative stress might be one physiological mechanism that promotes viral outbreaks and the occurrence of clinical signs. However, the relationships among oxidative stress, viral diseases and survival has poorly been addressed in wild animals. The aims of this study are to provide (i) the first data on the association among oxidative stress markers, clinical signs, and survival perspectives in a wild seabird and (ii) to assess experimentally whether the oral administration of antioxidants helps to recover from illness. To this end, we took advantage of a population of magnificent frigatebirds *Fregata magnificens*, a seabird breeding in French Guiana, whose chicks experience annually severe viral outbreaks likely due to an herpesvirus infection.

## Differences in acute phase response to bacterial, fungal and viral challenges in greater mouse-eared bats (*Myotis myotis*)

SELTMANN ANNE<sup>1,2</sup>, TROXELL SARA A<sup>1,2</sup>, SCHAD JULIA<sup>1</sup>, FRITZE MARCUS<sup>1,2</sup>, VOIGT CHRISTIAN C<sup>1,2</sup>, CZIRJÁK GÁBOR Á<sup>3</sup>

<sup>1</sup>Department of Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; seltmann@izw-berlin.de

<sup>2</sup>Institute of Biology, Freie Universität Berlin, Takustr. 3, 14195 Berlin, GERMANY

<sup>3</sup>Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY

The acute phase response (APR) is an evolutionarily well-conserved part of the innate immune defence. However, recent studies in bats yielded surprisingly diverse results compared to previous APR studies on both vertebrate and invertebrate species. This is especially interesting due to the known role of bats as reservoirs for zoonotic agents, especially for viruses. To better understand these discrepancies and the reservoir-competence of bats, we mimicked bacterial, viral and fungal infections in greater mouse-eared bats (*Myotis myotis*) and quantified APR over two days. Individuals reacted most strongly to fungal antigen (zymosan), reflected by increased numbers of neutrophils and lymphocytes at 48 hours post-injection (p.i.). In virus-challenged (PolyI:C) bats, we found increased neutrophil numbers 24 hours p.i., while in the bacterial (LPS) group, there were no differences in immune cell subtypes. Remarkably, none of the antigens elicited a fever response, change in body mass or in acute phase protein (haptoglobin) concentration. We add evidence to the hypothesis that some bat species may have adapted to tolerate intracellular (bacterial and viral) pathogens and show reduced inflammatory defences, which might be associated with their extraordinary role as reservoir hosts for zoonotic viruses.

## Seasonal variation in the spatiotemporal behavioural game between predators and prey

SIMON RICARDO NOUAILHETAS<sup>1</sup>, CHERRY SETH<sup>2</sup>, FORTIN DANIEL<sup>1</sup>

<sup>1</sup>Laval University, Centre d'Étude de la Forêt (CEF) and Biology Department, 1045 Avenue de la Médecine, pavillon Alexandre-Vachon, G1V 0A6, Quebec City, Quebec, CANADA; ricardo.simon.1@ulaval.ca

<sup>2</sup>Lake Louise, Yoho & Kootenay Field Unit, Parks Canada, Box 220, V0A 1M0, Radium Hot Springs, British Columbia, CANADA

The spatiotemporal game is a fundamental process governing the population dynamics of both predators and prey, with repercussions that can ripple through food webs. Prey attempt to minimise encounters with predators that strive to find prey. Such simple behavioural rules can nevertheless yield a vast range of game tactics contingent upon the characteristics of the players and their habitats. The effectiveness of a tactic at maximising fitness often reflects a cost-benefit trade-off in terms of where, when and how long an individual performs different activities, such as foraging. By regularly altering the settings under which the game is played, seasonal variation in abiotic and biotic attributes can shift costs and benefits, and lead to different tactics by both players. Yet, few studies have investigated seasonal plasticity in the spatiotemporal game played by the same populations of predators and prey. Here we assess the tactics deployed by GPS-collared plains bison (*Bison bison bison*) and grey wolves (*Canis lupus*) engaged in a spatiotemporal game in Prince Albert National Park, Canada, across three seasons. Responses to both the long-term seasonal space use (*i.e.* a pre-encounter tactic) and the real-time proximity of the opponent (*i.e.* a post-encounter tactic) were evaluated through habitat selection and movement analyses. Both species consistently selected meadows, which are rich forage patches for bison in the park, during any season. No pre- or post-encounter tactics by either player were detected from spring to mid-summer. By the second half of the summer and throughout the autumn, however, bison engaged in a shell game: both players made intensive use of the same meadows, but the relative odds of bison leaving meadows in under an hour increased as wolf late-summer and autumn seasonal use of those meadows also increased. In other words, bison still used the richest food patches but had shorter residency time where the local risk of encountering wolves was higher. As winter settled in, however, bison no longer pre-emptively abandoned meadows but generally moved away from wolves following an encounter, whereas wolves were able to select areas intensively used by their quarry across all habitat types. Our results demonstrate the highly dynamic nature of the spatiotemporal game between predators and prey, which reflect seasonal variations in player mobility, the availability of alternative prey and group characteristics determining prey vulnerability and predator effectiveness.

## **Scent-marking behaviour in grey wolves *Canis lupus* inhabiting managed forests in Northeast Poland**

STĘPNIAK KINGA M<sup>1</sup>, SZEWCZYK MACIEJ<sup>2</sup>, NIEDŹWIEDZKA NATALIA<sup>2</sup>, MYŚLAJEK ROBERT W<sup>2</sup>

<sup>1</sup>Warsaw University of Life Sciences, Faculty of Animal Sciences, Ciszewskiego 8, 02-786 Warszawa, POLAND; kinga.magdalena.stepniak@gmail.com

<sup>2</sup>University of Warsaw, Faculty of Biology, Institute of Genetics and Biotechnology, Pawińskiego 5a, 02-106 Warszawa, POLAND

Wolves live in family groups occupying vast territories. To avoid conflicts between individuals from neighbouring groups wolves apply territory marking using olfactory, visual and vocal signs. Marking with scats, urine and ground scratching play a particularly important role in maintaining territories, however, human caused alteration of the environment may modify marking behaviour. Earlier studies also suggested that wolf territories are marked mainly by the parental pair with the breeding male being the most active, while the offspring do not play an important role. Thus, we attempted to validate a hypothesis, that in managed forests wolves increase effectiveness of the scent-marking by depositing olfactory signs at intersections of roads, where the probability of being detected by other wolves is greatest. We further hypothesized, that marking is performed by all members of the wolf family group despite of its gender and social status.

We gathered data on wolf marking (130 markings with scats and urine) from December 2016 to March 2017 in managed forests of Northeast Poland. Altogether, we followed 388 km of forest roads, and checked junctions 436 times. Locations of scats and urinations were assessed with hand-held GPS. We also applied microsatellite genotyping using 13 polymorphic loci and two sex markers to identify individual wolves and their gender, and to exclude other species (i.e. dogs, foxes).

We found that wolves preferably deposit their scats and urinate at crossroads, mainly on conspicuous substrates. More scats were found at easily passable intersections. Scats were deposited by various members not only by the dominant pair. We also found that males deposit more scent-markings than females.

We confirmed that for wolves, similarly like for other canids, crossroads play an important role in the facilitation of the olfactory communication. Our studies also highlighted an importance of other members of the family groups in the maintenance of the territory.

## **Secrets of success in a landscape of fear: Urban mammals adjust risk perception and tolerate disturbance**

STILLFRIED MILENA, GRAS PIERRE, BÖRNER KONSTANTIN, GÖRITZ FRANK, PAINER JOHANNA, RÖLLIG KATHLEEN, WENZLER MORITZ, HOFER HERIBERT, ORTMANN SYLVIA, KRAMER-SCHADT STEPHANIE

<sup>1</sup>Leibniz Institute for Zoo-and Wildlife Research, Department of Evolutionary Ecology, Alfred-Kowalkestraße 17, 10314 Berlin, GERMANY; stillfried@izw-berlin.de

<sup>2</sup>Leibniz Institute for Zoo-and Wildlife Research, Department of Reproduction Management, Alfred-Kowalkestraße 17, 10314 Berlin, GERMANY

<sup>3</sup>Humboldt-University, Geography Department, Rudower Chaussee 16, 12489 Berlin, GERMANY

<sup>4</sup>Berlin-Brandenburg Institute of Advanced Biodiversity Research, Altensteinstr. 34, 14195 Berlin, GERMANY

In urban areas with a high level of human disturbance, wildlife has to adjust its behaviour. This so called ‘landscape of fear’ effect can be studied in risk perception during movement in relation to specific habitat types; whereby individuals trade off foraging time and hiding to avoid disturbance. Due to its high behavioural plasticity and increasing occurrence in urban environments, wild boar (*Sus scrofa*) is an excellent model organism to study adjustment to urbanisation. With the help of GPS tracking, space use of 11 wild boars from Berlin’s metropolitan region was analysed: we aimed at understanding how animals adjust space use to deal with the landscape of fear in urban areas compared to rural areas. We compared space use vs. availability of 11 landscape types with help of generalized linear mixed models. First, we studied landscape types selected by rural versus urban wild boar, second, we analysed distances of wild boar locations to each of the landscape types. Finally, we mapped the resulting habitat selection probability to predict hotspots of human-wildlife conflicts.

A higher tolerance to disturbance in urban wild boar was shown by a one third shorter flight distance and by an increased re-use of areas close to the trap. The space use vs. availability approach revealed that urban wild boar had a strong preference for natural landscapes such as swamp areas, green areas and deciduous forests, and areas showing a high NDVI (normalized difference vegetation index). The areas selected by urban wild boar were often located closely to roads and houses, while rural wild boar selected similar areas far away from roads and close to agriculture. The spatial distribution maps show that a large area of Berlin would be suitable for urban wild boar but not their rural conspecifics, with the most likely reason being a different perception of anthropogenic disturbance. Wild boar therefore showed considerable behavioural plasticity suitable to adjust to human-dominated environments in a potentially evolutionarily adaptive manner.

## **Biochemical and mineral blood profile of Western Derby eland in the conservation breeding population in Senegal**

STOKLASOVÁ LUCIE<sup>1</sup>, HEJCMANOVÁ PAVLA<sup>1,2</sup>

<sup>1</sup>Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýcká 129, Prague - Suchdol, CZE 165 00, CZECH REPUBLIC

<sup>2</sup>Derbianus Conservation, Kamýcká 129, Prague – Suchdol, CZE 165 00, CZECH REPUBLIC; hejcmanova@derbianus.cz

The Western Derby eland (*Taurotragus derbianus derbianus*, WDE) is a critically endangered antelope with less than 200 individuals in the wild, living in Niokolo Koba National Park in Senegal. One of the potential and unexplored factors of low population size is an inappropriate nutritional status and mineral deficiencies of animals in the wild. Our aim was to determine biochemical parameters and minerals (Cu, Fe, Zn, Se, K, S) in serum of WDEs in the semi-captive conservation breeding population in two nature reserves in Senegal as a baseline for future investigation of wild animals. Blood samples were collected from 11 WDEs (6 males and 5 females) during animal transports in Bandia and Fathala reserves in Senegal in March 2017. Biochemical parameters were determined by IDEXX VetTest Chemistry Analyser, Fe, S, Se and Zn were analysed by Inductively coupled plasma atomic emission spectroscopy (ICP-OES), Cu by Graphite furnace atomic absorption spectroscopy (GF-AAS) and K by Flame atomic absorption spectroscopy (AAS-flame) and tested with Mann-Whitney U tests for differences between sex, age classes (1 and 2 years old), and reserves.

The majority of biochemical and mineral parameters were within the range of reference values listed for the Eastern subspecies of Giant eland (synonymous to Derby eland) in Zoological Information Management System (ZIMS). There were no differences in any parameter between age classes, but differences in the sexes were detected for cholesterol, Mg and lactic acid which were higher in females than in males and between the reserves for urea, glucose, creatine kinase, Fe, K and S which were higher in the Bandia than the Fathala reserve. Only glucose was higher in the Fathala than Bandia reserve. As the majority of parameters corresponded to physiological values, there is no indication for potential nutritional and mineral deficiencies within conservation breeding program in reserves. Thus the problem of low reproduction rate has probably different causation.

The study was supported by Czech University of Life Sciences Prague, grant CIGA 20175004.

## Endocrinological insights into the European badger's (*Meles meles*) female reproductive cycle: an example from a high density population (Wytham Woods, UK)

SUGIANTO NADINE ADRIANNA<sup>1</sup>, HEISTERMANN MICHAEL<sup>2</sup>, MALO AURELIO<sup>3</sup>, NEWMAN CHRIS<sup>1</sup>, WALKER SUSAN<sup>4</sup>, MACDONALD DAVID WHYTE<sup>1</sup>, BUESCHING CHRISTINA DAGMAR<sup>1</sup>

<sup>1</sup>Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Recanati-Kaplan Centre, Abingdon Road, Tubney House, Tubney, Oxfordshire OX13 5QL, UK; nadine.sugianto@zoo.ox.ac.uk

<sup>2</sup>Endocrinology Laboratory, German Primate Center, Kellnerweg 4, 37077 Göttingen, GERMANY

<sup>3</sup>Department of Zoology, University of Oxford, The Tinbergen Building, South Parks Road, Oxford OX1 3PS, UK

<sup>4</sup>North of England Zoological Society, Chester Zoo, Caughall Road, Upton-by-Chester CH2 1LH, UK

European badgers (*Meles meles*) undergo delayed implantation (up to 330 days), and superfoetation. Here we characterise changes in circulating female sex-steroid levels during and after delayed implantation in a high-density badger population in Wytham Woods (Oxfordshire-UK). Using Enzyme-Immuno-Assays, we analysed seasonal progesterone, oestradiol, and oestrone titres in single blood serum samples of 97 wild females. Blood samples were collected under sedation to coincide with key-stages of badger reproductive biology: (1) in January (winter) during the first pregnancy trimester and start of the main mating season, (2) in May/June (spring) at the end of the main mating season and cubs weaning, (3) in August (summer) during lowest food abundance, and (4) in November (autumn) during reproductive quiescence when badgers reach their maximum body weight. Oestradiol levels were highest in spring in females that had not raised cubs ( $119.97 \pm 139.34$  pg/ml), dropped sharply in summer ( $26.85 \pm 10.01$  pg/ml), and remained low ( $34.93 \pm 12.17$  -  $44.00 \pm 18.37$  pg/ml) during the following seasons. Oestrone levels were high in spring ( $66.09 \pm 20.51$  -  $77.00 \pm 14.53$  pg/ml), dropped during summer ( $32.62 \pm 21.91$  pg/ml), peaked in autumn ( $87.94 \pm 9.80$  pg/ml), and remained elevated for pregnant females in winter ( $82.55 \pm 46.04$  pg/ml). Progesterone was low ( $0.32 \pm 0.23$  -  $0.47 \pm 0.08$  ng/ml) throughout all seasons, but increased significantly during winter pregnancy ( $1.34 \pm 0.38$  ng/ml). Oestrone levels always exceeded oestradiol levels, but this difference was statistically significant in spring only in females that had not lactated ( $p = 0.021$ ), in all females in summer ( $p = 0.008$ ) and autumn ( $p < 0.001$ ), and in pregnant females in winter ( $p = 0.002$ ). Thus, oestrone levels likely sustain preimplanted blastocysts during delayed implantation, while oestradiol governs oestrus during the main mating season. Our oestradiol data indicate that females in this high density study population likely exhibit only one oestrus, because we could find no endocrinological evidence for additional oestrus periods later in the year.

## Detection of IGF-I concentrations in saliva from red deer (*Cervus elaphus*)

TALLO-PARRA ORIOL<sup>1</sup>, KÖHLER MEIKE<sup>2</sup>, CARBAJAL ANNAIS<sup>1</sup>, ARNOLD WALTER<sup>3</sup>, STALDER GABRIELLE<sup>3</sup>, GERRITSMANN HANNO<sup>3</sup>, LOPEZ-BEJAR MANEL<sup>1</sup>

<sup>1</sup>Universitat Autònoma de Barcelona, Faculty of Veterinary, Department of Animal Health and Anatomy, Campus de la UAB, 08193, Bellaterra (Barcelona), SPAIN; manel.lopez.bejar@uab.cat

<sup>2</sup>Institut Català de Paleontologia Miguel Crusafont (ICP), Campus de la UAB, 08193, Bellaterra (Barcelona), SPAIN

<sup>3</sup>Wildlife Medicine and Applied Ecology, Research Institute of Wildlife Ecology, University of Veterinary Medicine (Vetmeduni Wien), Vienna, AUSTRIA

The insulin-like growth factor 1 (IGF-I) is a growth factor involved in numerous growth and differentiation processes. The practical benefits of saliva matrix for hormonal analyses have been widely known, especially in research involving wildlife species. Saliva collection is minimally invasive, easy to perform and fast. Moreover its sampling is safer than blood sampling for the animal and the staff. Thus, the aim of this study was to assess whether the detection of IGF-I from red deer (*Cervus elaphus*) could be achieved in saliva samples using an IGF-I ELISA kit. Benefiting from a long-term study, saliva and blood samples were collected from four red deer (three males and one female) of ages ranging from 3 to 361 days old. Overall, 15 saliva samples and 24 serum samples were analysed. After collection, saliva samples were frozen at -20 °C immediately and serum samples were also frozen after centrifugation. A cow IGF-I sandwich ELISA kit (LSBio, LifeSpan BioSciences, Inc., Seattle, WA, USA) with a sensitivity of 0.0289 ng/ml was used for the detection of IGF-I concentrations, obtaining a CV intraassay of 4.4 %. IGF-I was detected in all serum samples and in 12 out of 15 saliva samples. Average immunoreactive IGF-I concentrations were  $0.77 \pm 1.64$  ng/ml in saliva and  $20.93 \pm 9.07$  ng/ml in serum ( $p < 0.0001$ ). IGF-I concentrations in saliva were lower than in serum, in a similar ratio that has been described in humans, suggesting that saliva could reflect the unbound bioactive hormone level. No correlations were detected between salivary and serum IGF-I concentrations. No effects of sex, age or body mass/food intake ratio on salivary or serum IGF-I concentrations were detected. This study provides for the first time the evidence of salivary IGF-I in red deer and opens the possibility of future work for the establishment of bioactive IGF-I analysis in saliva as a practical tool for research in growth and metabolism endocrinology.

## **Global warming impacts on hibernation of garden dormouse (*Eliomys quercinus*)**

TARMANN IRIS, NOWACK JULIA, RUF THOMAS, GIROUD SYLVAIN

Department of Integrative Biology and Evolution, University of Veterinary Medicine, Vienna, Savoyenstraße 1, Vienna 1160, AUSTRIA; iris.tarmann@students.boku.ac.at

Torpor is an energy saving strategy achieved by substantial reductions of metabolic rate (MR) and body temperature (T<sub>b</sub>) to survive periods of low food and/or water availability. Previous studies showed that the rate of periodic rewarming from torpor is associated with a decrease of telomere lengths. Telomere shortening is traditionally used as a proxy for physiological aging and is directly impacted by the production of reactive oxygen species during phases of high MR, such as experienced during periodic arousals. Increasing temperatures during winter could have an effect on both energy savings and telomere changes associated with hibernation. To determine if mild winters have an impact on these two aspects, we compared torpor patterns of garden dormice (*Eliomys quercinus*), a small, hibernating rodent, kept at controlled temperatures of either 15 °C or 4 °C. We assessed body mass loss and sampled DNA, via buccal swabs, for analyses of relative telomere length (RTL) at four time points during winter (at onset and at emergence from hibernation and at two time-points during hibernation). Our results show that animals hibernating at the warmer temperature spent significantly less time torpid and experienced more and longer arousals than animals hibernating at 4 °C. Additionally, body mass loss was significantly greater in those animals. Although RTL significantly increased over the course of hibernation in both groups, we found a significant higher increase among individuals experiencing warmer temperatures. Importantly, our data suggest that garden dormice are able to elongate their telomeres, presumably during periods of normothermic T<sub>b</sub>. Although hibernation at warmer temperatures has a positive effect on telomere length, hence physiological aging, our data clearly show that hibernating at higher temperatures causes an additional energy expenditure which increases the risk to entirely deplete fat reserves before natural emergence from hibernation.

## **Grouse and wind energy: pointing dogs in conservation research**

TAUBMANN JULIA<sup>1,2</sup>; HÖRNELL-WILLEBRAND MARIA<sup>3</sup>; ANDRÉN HENRIK<sup>4</sup>

<sup>1</sup>University of Freiburg, Chair of Wildlife Ecology and Management, GERMANY;  
julia.taubmann@wildlife.uni-freiburg.de

<sup>2</sup>Forest Research Institute of Baden-Württemberg, Wildlife Ecology Group, GERMANY

<sup>3</sup>Naturvårdsverket, Swedish Environmental Protection Agency, Enheten for viltanalys, SWEDEN

<sup>4</sup>Grimsö Wildlife Research Station, Swedish University of Agricultural Science, SWEDEN

The international research project “Capercaillie & Wind Energy” studies the possible effects of wind turbines on capercaillie in several areas using the “Multiple-Before-After-Control-Impact” (MBACI) design. Differentiated research methods are used to assess the influence of wind turbines on spatio-temporal behaviour, stress physiology, reproduction and predation of this forest grouse. It is possible for researchers to locate the study species and their indirect signs, but by using professional detection dogs the probability of finding present birds highly increases and thus makes it more effectively. The use of pointing dogs for wildlife monitoring and research is predominantly known in countries with a tradition of hunting, such as Italy, Great Britain and Scandinavia. Counting grouse using trained pointer dogs has been shown to produce consistent, reliable density estimates and is routinely used by reserve managers and scientists alike. Furthermore, pointing dogs allow researchers to find and count broods systematically over large scales. However, the methods require well-trained dogs and experienced observers. In this project, the reproduction success of capercaillie and black grouse will be estimated in areas with and without wind turbines using pointing dogs. In late summer, transects will be followed in randomly selected grids. By investigating more than one area, different local situations can be taken into account and generally applicable results generated. This will allow for statements about single occurrences valid on more than just small scales. The individuals will be counted along transects and classified as cock, hen or chick. Additionally, working dog performance will be studied using high resolution GPS data, climate data and individual data characterising the dogs.

## **DOES size matter? Characterisation of factors influencing male reproductive success in the Eastern grey kangaroo (*Macropus giganteus*)**

THOMAS GEORGIA<sup>1</sup>, ELDRIDGE MARK<sup>2</sup>, GRUEBER CATHERINE<sup>1,3</sup>, MACHOVSKY-CAPUSKA GABRIEL<sup>1</sup>, SPIELMAN DEREK<sup>4</sup>, HERBERT CATHERINE<sup>1</sup>

<sup>1</sup>School of Life and Environmental Sciences, Faculty of Science, Level 5, Carslaw Building, The University of Sydney, NSW 2006, AUSTRALIA ; georgia.thomas@sydney.edu.au

<sup>2</sup>Australian Museum Research Institute, Australian Museum, 1 William Street, Sydney, NSW 2010, AUSTRALIA

<sup>3</sup>San Diego Zoo Global, 15500 San Pasqual Valley Rd, Escondido, CA 92027, USA

<sup>4</sup>Faculty of Veterinary Science, The University of Sydney, Regimental Dr, Camperdown, NSW 2050, AUSTRALIA

The Eastern grey kangaroo (*Macropus giganteus*) is one of the largest existing marsupials. It exhibits a polygamous mating system, established social structuring, and sexual size dimorphism. In this context, males are expected to exhibit traits that influence their ability to successfully reproduce. In eutherian species, these can include weaponry, a larger body size and/or higher testosterone concentrations. Limited research has been performed on marsupials, impeding the development of evolutionary theories surrounding this extant mammalian lineage. A study by MILLER *et al.* (2010) concluded that body size, testosterone, relatedness and dominance status influenced reproductive success in captive populations. However, a more recent study on south-eastern Australian free-ranging kangaroos by RIOUX-PAQUETTE *et al.* (2015) discovered a lack of mating skew towards certain males, thus concluding that females were not monopolised each breeding season. The present study therefore aimed to identify factors influencing male reproductive success in a free ranging New South Wales Eastern grey kangaroo population.

Approximately 200 free ranging Eastern grey kangaroos reside on the Nelson Bay Golf Course, approximately 1 km<sup>2</sup> in size; the research area for this study. Of these, 156 have been captured, uniquely tagged and had tissue, morphometric measurements and faecal samples collected. Candidate breeding male (over 35 kg) body weight, skeletal size, testes size, testosterone and glucocorticoid concentrations, relatedness, parasite load and level of association between potential mates were investigated as influential factors of male siring success. Paternity was assigned to 121 offspring across four breeding seasons (2012 - 2015), to measure male reproductive success.

At least half of the candidate males sired offspring each season, with certain males consistently siring significantly more offspring each season than conspecifics. In particular, body size (as indicated by body weight, leg length, testes size and testosterone) significantly influenced male reproductive success. Foot length was not correlated with body size and showed no significant relationship with siring success. Similarly, glucocorticoid concentration held no significant relationship. In addition, findings related to parasitism, relatedness and level of association between sires and dams will be discussed during the presentation. All results will also be discussed within the context of other studies on this species at varying latitudes. Information found in this study will contribute to the development of broad evolutionary theories across all extant mammalian lineages, as well as providing valuable information to aid the management of wild and captive populations.

MILLER EJ, ELDRIDGE MDB, COOPER DW, HERBERT CA (2010): Dominance, body size and internal relatedness influence male reproductive success in Eastern grey kangaroos (*Macropus giganteus*). *Reprod Fertil Dev* **22**, 539 - 549.

RIOUX-PAQUETTE E, GARANT D, MARTIN AM, COULSON G, FESTA-BIANCHET M (2015): Paternity in Eastern grey kangaroos: moderate skew despite strong sexual dimorphism. *Behav Ecol* **26**, 1147-1155.

## Identification of putative alarm pheromones in albino mice exposed to predator stress

RENGARAJAN ANANTHA NAYAKI<sup>1,2</sup>, KRISHNAN MANISH<sup>1</sup>, BALAMUTHU KADALMANI<sup>2</sup>, GOVINDHA RAJU ARCHUNAN<sup>2</sup>, PETER SUBHASH<sup>1</sup>

<sup>1</sup>Center for Evolutionary and Integrative Biology, University of Kerala, Kariavatom, Thiruvananthapuram 695581, Kerala, INDIA; subhashpeter@yahoo.com

<sup>2</sup>Department of Animal Science, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, INDIA; archunan@bdu.ac.in

Chemical signal plays an enormous role in the interaction of many mammalian species by means of chemical communication in their social lives especially in territory marking and for the avoidance of danger from a predator. Mice release a specific odor when they are under stress or in danger which makes alarm signals. These molecules warn their conspecifics for an alarm indication as its benefits to their kin to escape from the danger by altering the behaviors. In order to understand the anxiety-related molecules that cause stress-related behavior, we examined the influence of a natural predator, *Felis catus*, on the behaviour of albino mice. The stress was provoked by the direct contact of *Felis catus* (predator) to the mice performed in glass tank separated by mesh wire by which the *Felis catus* predator could reach the other side with its paws. This developed the stress in mice (prey) to undergo behavioural changes and also evoke a fear response which generates an alarm signal through urine and faeces to protect their kin from threat. This excellent paradigm provides an opportunity to determine alarm pheromone as chemical cues in their urine and feces in mice upon stress challenges. After sequential analysis of the stress-related behaviours, we found that certain chemical compounds in the urine and feces are a multi component, with two or more compounds produced from different glands which simultaneously alert and evoke anxiogenic properties in mice. Furthermore, we performed light-dark box plus elevated maze experiments and investigated the changes in the anxiety-like behavior. We identified stress-related molecules that could act as alarm pheromone and initiate changes in their behaviour by inducing anxiety and stress. Further, it appears that these chemical signals alert the conspecifics and initiate alarm communication among them.

## **Where are you and if so how many? - European wildcats as example for assessing conservation status of rare and elusive species**

TIESMEYER ANNIKA<sup>1,2</sup>, STEYER KATHARINA<sup>1</sup>, MÖLICH THOMAS<sup>3</sup>, VOGEL BURKHARD<sup>3</sup>, NOWAK CARSTEN<sup>2</sup>, BALZER SANDRA<sup>1</sup>

<sup>1</sup>Federal Agency for Nature Conservation (BfN), section zoological species conservation, Konstantinstr. 110, 53179 Bonn, GERMANY; Annika.tiesmeyer@bfm.de

<sup>2</sup>Senckenberg Research Institute and Natural History Museum, Conservation Genetics Group, Clamecystr. 12, 63571, Gelnhausen, GERMANY;

<sup>3</sup>BUND für Umwelt und Naturschutz Deutschland (BUND), Landesverband Thüringen e.V., Landesgeschäftsstelle, Trommsdorffstraße 5, 99084 Erfurt, GERMANY

Monitoring of endangered species is essential for planning and evaluating conservation measures. The monitoring of rare and elusive species or species occupying large home ranges is complex and time-consuming. Ongoing technological advance in genetic analysing of non-invasive samples, as hair, scat or urine, enables to develop standardized and systematic sampling methods as hair traps or scat surveys. However, there is still a lack of knowledge on the required spatial and temporal sampling effort needed to obtain solid data on the conservation status, distribution or population sizes of some species.

European wildcats (*Felis silvestris*) belong to the most intensively and comprehensively studied carnivores in Central Europe. In 2012 the project “wildcat leap” was initiated by the BUND (Friends of the Earth Germany e.V.) funded by the Federal Agency of Nature Conservation and the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety in the frame of the National Programme on Biological Diversity. More than 1.700 hair traps treated with Valerian as attractant were inspected across Germany with the support of 750 volunteers over several years and resulted in 3008 detections of wildcats. At 16 locations sampling was conducted along a systematic and standardized grid approach of each 50 cells a 9 km<sup>2</sup> equipped with two hair traps. These empirically assessed fine scale data allowed for testing optimal schemes of spatial and temporal monitoring effort for assessing distribution and population sizes. Distribution was assessed using positive and negative detection events. Population sizes were estimated based on the capture history of individuals in the study area using spatially explicit capture recapture (SECR) software.

For assessing distribution, 10 weekly inspections of hair traps with a density of 10 per 100 km<sup>2</sup> showed statistically reliable results. A higher effort was required for estimating population sizes with 10 weekly inspections of hair traps and a density of 50 per 100 km<sup>2</sup> to achieve robust estimations with low confidence intervals. Our results suggest monitoring systems should be adapted, depending on the question to be answered and to account for statistical power of the data. In the future these findings can be applied to plan and optimize assessments of the conservation status of wildcats and other rare and elusive species.

## **Data for a Data Deficient Species: The Annamite striped rabbit (*Nesolagus timminsi*) of Vietnam and Laos**

TILKER ANDREW; NGUYEN AN THE TRUONG; ABRAMS JESSE; WILTING, ANDREAS

Leibniz Institute for Zoo and Wildlife Research, Junior Research Group: Biodiversity and Biogeography of Southeast Asia, Alfred-Kowalke-Straße 17, 10315 Berlin, GERMANY; tilker@izw-berlin.de.

The Annamite striped rabbit (*Nesolagus timminsi*) is a forest-dwelling lagomorph endemic to the Annamite mountains of Vietnam and Laos. The species was only described by science in 2000 (AVERIANOV *et al.*, 2000), and little is known about it, including its basic ecology and conservation status. It is currently listed under the IUCN Red List of Threatened Species as Data Deficient. We used a coarse and fine grid systematic camera trapping design to study the species in four protected areas in central Vietnam and southeastern Laos. In 29,180 nights of camera trapping effort, we recorded 152 independent events ( $\Delta = 60$  min) at 36 out of 266 different camera trap stations, representing the largest and most complete dataset on the species to date. Here we provided the first overview about the daily activity pattern of Annamite striped rabbits. Furthermore, we use single-species occupancy models on the coarse grid data to estimate occupancy and assess which habitat and anthropogenic factors influence the occupancy of the species. We use N-mixture models for the fine grid data, where a paired camera-trapping setup allowed individual identification, to estimate local abundance. This study is the first comprehensive work on the threatened Annamite striped rabbit. By understanding its basic ecology, and gaining insight into anthropogenic factors that influence its occupancy and abundance, we provide information useful for targeted conservation efforts for this little-known Annamite endemic species.

AVERIANOV AO, ABRAMOV AV, TIKHONOV AN (2000): A new species of *Nesolagus* (Lagomorpha, Leporidae) from Vietnam with osteological description. Contributions from the Zoological Institute, St. Petersburg **3**, 1-22.

## ***Yersinia enterocolitica* isolates from wild boar hunted in North-western Italy: presence of genes of pathogenicity and antibiotic-resistance**

VENCIA WALTER, MODESTO PAOLA, LAZZARA FABRIZIO, PUGLIANO MARIA CONCETTA, ERCOLINI CARLO, SERRACCA LAURA, DELLEPIANE MONICA, AROSSA CLAUDIO, MIGNONE WALTER, DECASTELLI LUCIA, ADRIANO DANIELA, ZOPPI SIMONA, DONDO ALESSANDRO, RAZZUOLI ELISABETTA, FERRARI ANGELO

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Turin, ITALY; paola.modesto@izsto.it

*Yersinia enterocolitica* are zoonotic bacteria able to infect humans and animals, recognized as the third cause of foodborne disease in Europe. These bacteria are often isolated in swine and wild boar: recent papers showed a prevalence of 3.5 % in European wild boar. However, data on their pathogenicity and the contribution to the spread of resistance to antibiotics (AR) are still lacking. This is particularly of concern in highly urbanized areas like Liguria Region where increased human-wildlife interface facilitates the spread of infectious disease from wildlife to domestic animals and humans. The aim of our study was to investigate the presence of *Y. enterocolitica* in wild boar hunted in Liguria from 2013 to 2017 and evaluate the presence of genes of pathogenicity (GoP) and AR.

Liver samples (n = 4282) were analysed according to ISO10273-2003 and the isolated strains were biotyped and serotyped. RT-PCR was used to assess virulence genes presence: ail (attachment and invasion locus), ystA (Yersinia stable toxin), ystB, inv (invasin), myfA (mucoid Yersinia factor) and ymoA (Yersinia modulator). The strains were tested by Kirby-Bauer method to verify sensitivity to: Ampicillin, Chloramphenicol, Ceftiofur, Enrofloxacin, Gentamicin, Kanamycin, Streptomycin, Sulfisoxazole, Triple-Sulfa, Sulfamethox+Trime-Thoprim, Tetracycline.

2.9 % out of 126 samples resulted positive. Through biotyping we predominantly found *Y. enterocolitica* 1A (91.8 %) followed by 1B (7.2 %) and 2 (1 %). Most isolated strains resulted O-non typeable (44.3 %); 1A strains belonged to serotypes O:1.2 (4.1 %), O:3 (7.2 %), O:5 (11.3 %), O:8 (30.9 %), O:9 (3 %), and 1B to serotypes O:1.2, O:5, O:8. Most strains were included in serotypes O:5 and O:8, the most relevant serotypes causing gastroenteritis in humans in Europe. Concerning the GoP, we observed the presence of ystB in 59.3 % of the strains followed by ymoA (42.2 %), ail (35.2 %), ystA (16.4 %), myfA (10.2 %), inv (7.8 %). The biotype 1A strains have been always considered non-pathogenic, since they do not have pYV plasmid and some chromosomal virulence genes. In our study, we demonstrated that 1A strains carry other virulence genes (ystA, ail, ystB, ymoA and myfA).

The AR profiles evidenced that 92 % of the strains was resistant to Ampicillin, followed by Sulfisoxazole (24.8 %), Triple-Sulfa (22.3 %), Ceftiofur (7.4 %), Gentamicin (1.6 %), Kanamycin (1.6 %), Streptomycin (0.8 %), Tetracycline (0.8 %).

These results indicate that *Y. enterocolitica* 1A could potentially infect humans and animals. Other studies are needed to demonstrate our hypothesis and the ability of such strains to invade and modulate gut immune response. Moreover, this study showed the existence and diffusion of multi-resistance of *Y. enterocolitica* in the Ligurian wild boar.

## **Getting to the fat of the matter: The consequences of supplementing garden birds with fat-rich food**

VOIGT-HEUCKE SILKE L<sup>1,2</sup>, SCHLAG LENA<sup>1,2</sup>, VOIGT CHRISTIAN C<sup>2</sup>, LANDGRAF CONNY<sup>1,2</sup>, CYRACH MELANIE<sup>1</sup>, KIEFER SARAH<sup>1,2</sup>, VON KEYSERLINGK JENNIFER<sup>1,3,4</sup>, KIPPER SILKE<sup>1,5\*</sup>, WEISS MICHAEL<sup>1,6\*</sup>

\*both authors contributed equally to the manuscript

<sup>1</sup>Animal Behaviour, Institute of Biology, Freie Universität Berlin, Takustr. 6, 14195 Berlin, GERMANY; voigt.heucke@googlemail.com

<sup>2</sup>Leibniz Institute for Zoo and Wildlife Research, Department for Evolutionary Ecology, Alfred Kowalke Str. 17, 10315 Berlin, GERMANY

<sup>3</sup>Department of Ecology (Ecohydrology and Landscape Evaluation), Technische Universität Berlin, Ernst-Reuter-Platz 1, 10587 Berlin, GERMANY

<sup>4</sup>Institute of Earth and Environmental Sciences, University of Potsdam, Karl-Liebkecht-Str. 24-25, 14476 Potsdam-Golm, GERMANY

<sup>5</sup>Department of Sociobiology/Anthropology, Georg-August-Universität Göttingen, Kellnerweg 6, 37077 Göttingen, GERMANY

<sup>6</sup>Department of Exposition, Unit Epidemiology, Statistics and Mathematical Modelling, Federal Institute for Risk Assessment (BfR), Max-Dohrn-Str.8-10, 10589 Berlin, GERMANY

Although supplementing the diet of garden birds with fat-rich food is a common practice in the northern hemisphere, its beneficial effect on reproduction is controversially discussed. We provided fat balls to breeding blue and great tits on an experimental area and no supplementary food to birds of a control area. Further, we added <sup>15</sup>N-enriched glycine to the fat balls to infer the amount of supplemented food that was assimilated by parent birds and the amount that they fed to their chicks. We demonstrate that adult birds fed on the provisioned food, and parent birds also provided their chicks with supplemented food. We found a hatching failure rate of 47 % of all nests on the supplemented area, in contrast to only 13 % of all nests on the control area. The hatching rate for blue tit nests decreased with the amount of fat consumed by mother birds. However, chicks of the experimental area that had fledged were larger and heavier than those of the control area. Our results indicate that feeding fat-rich food during reproduction of birds may drastically reduce the breeding success of garden birds, yet it might enhance the development of chicks in large broods.

## **The masked invaders of Berlin: Movement ecology, behaviour and diet of raccoons along a rural-urban gradient**

WEH CAROLIN, BERGER ANNE, HOFER HERIBERT, KRAMER-SCHADT STEPHANIE, ORTMANN SYLVIA

Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Str. 17, 10315 Berlin, GERMANY; weh@izw-berlin.de

Nowadays, 54 % of the human world population is living in cities, which already sum up to 5 % of the planet's landmass. This trend was increasing by 1.95 % during the last 5 years. This urban sprawl, associated with habitat fragmentation, traffic increase, soil sealing, permanent presence of humans and therefore noise, light and air pollution requires an enormous adaptability of the fauna inhabiting urban areas. On the other hand, these presumably negative impacts might be alleviated by positive factors such as the absence of predators, higher temperatures or surplus food in terms of anthropogenic waste.

Up to now there is not much knowledge about the adaptation mechanisms of wildlife to life in the city. Hence, the Leibniz Institute of Zoo and Wildlife Research (IZW) started a multispecies approach to study the adaptation of wildlife to urbanisation. Within the Bridging in Biodiversity Science (BIBS) Project we study the entire chain of causes, mechanisms and consequences of rapid changes across different ecosystems and spatio-temporal scales. One of the three main foci of this interdisciplinary project is the ecology of wildlife along a rural-urban gradient.

Within this framework, we study raccoons (*Procyon lotor*), a wildlife species currently invading the city of Berlin. Raccoons are one of the most successful new arrivals among German wildlife species and now even officially declared to be "invasive" (European Commission Nr. 1143/2014). This makes them an excellent model to study invasion processes and the means by which omnivores successfully adjust to novel habitat such as the urban space.

In this project we will investigate space use, movement behaviour, diet and genetic structure of the Berlin raccoon population. We will capture raccoons on different sites along an urban rural gradient and equip them with GPS collars also containing 3D-acceleration sensors to detect different behaviours of the individuals. Faecal samples will be collected to assess the diet of the raccoons by means of metabarcoding.

Apart from understanding adjustment mechanisms of urban wildlife, we will use the results to optimise wildlife management in cities.

## Use of animal-protein free extenders as alternatives to standard egg-yolk based extender in semen cryopreservation of the black rhino (*Diceros bicornis*) and Indian rhino (*Rhinoceros unicornis*)

WOJTUSIK JESSYE, STOOPS MONICA A, ROTH TERRI L

Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, 3400 Vine St., Cincinnati, OH, 45220, USA; jessye.wojtusik@cincinnati-zoo.org

African black (*Diceros bicornis*) and Indian rhinos (*Rhinoceros unicornis*) are critically endangered and vulnerable, respectively. To preserve genetic diversity and facilitate captive breeding, semen cryopreservation techniques were developed using extenders containing the animal-protein egg-yolk. Efficacy of such media is questioned as inter-batch composition varies and there is risk of bacterial and viral contamination. The goal of this study was to test animal-protein free extenders. Semen was collected via electroejaculation from ten rhinos (six black, four Indian). Control samples were diluted 1:1 with established semen extender (EQ; containing lactose, disodium EDTA, egg yolk, glucose, Equex STM, penicillin, streptomycin), whereas for treatment groups, egg yolk was replaced with 1 % or 2 % soy lecithin (1 % SL; 2 % SL), coconut milk (CM), or coconut water (CW). Samples were cooled in a water bath to 4 °C then diluted step-wise 1:1 to a final 5 % glycerol concentration, equilibrated for 1 h, loaded into 0.5 ml straws, lowered into a charged dry shipper for 10 min and then plunged into LN<sub>2</sub>. Samples thawed for 10 s at RT then 37 °C for 20 s were evaluated for sperm motility, viability, morphology, progression, and acrosomal integrity at 0, 1, 3, 6 and 24 h post-thaw. Data were analysed using repeated measures GLM and presented as means ± SD. Post collection motility was 84.5 ± 7.6 %, progression: 3.6 ± 0.6 (scale 0 - 5), viability: 71.3 ± 6.9 %, intact acrosomes: 83.4 ± 7.1 % and morphologically normal spermatozoa: 78.8 ± 13.6 %. Post-thaw motility and progression decreased ( $p < 0.05$ ) in all groups (EQ: 58.5 ± 14.2 %, 2.7 ± 0.7; 1 % SL: 28.4 ± 14.6 %, 1.8 ± 0.6; 2 % SL: 31.8 ± 18.6 %, 1.9 ± 0.8; CM: 0 ± 0 %, 0 ± 0; CW: 0 ± 0 %, 0 ± 0), were lower in all treatments than control and decreased over time ( $p < 0.05$ ). Motility and progression did not differ between 1 % SL and 2 % SL, but were lower ( $p < 0.05$ ) in CM and CW treatment groups, and acrosomal integrity was higher ( $p < 0.05$ ) in EQ, 1 % and 2 % SL than CM and CW, over time. Viability was greatest in EQ and 2 % SL followed by 1 % SL, then CM and CW ( $p < 0.05$ ) post-thaw. Morphology did not differ among treatments and morphology, acrosomal integrity, and viability did not change over time ( $p > 0.05$ ). Although some sperm did survive cryopreservation in 1 % SL and 2 % SL, animal-protein free treatments were inadequate substitutes for egg-yolk based extenders for rhino semen cryopreservation.

## **Food habits and diet of foxes (*Vulpes vulpes*) during period they feed their cubs in the wild (Kamchatka, Russia)**

YACHMENNIKOVA ANNA A<sup>1,2</sup>, BLIDCHENKO EKATERINA Y<sup>3</sup>

<sup>1</sup>AN Severtsov Institute of Ecology and Evolution, Russian Academy of Science, Leninsky pr. 33, 119071, Moscow, RUSSIA; felis.melanes@gmail.com

<sup>2</sup>Kronotsky Nature Reserve, Ryabikova Street, 48, Yelizovo, Kamchatka region, 684000, RUSSIA

<sup>3</sup>Land of the Leopard National Park, pr. 100-letiya Vladivostoka, 127, Vladivostok, Primorskiy kray, 690068, RUSSIA

The nutrition and composition of the foraging base of carnivorous mammals at different stages of the life cycle is one of the main aspects of their ecology. The work took place in the Kronotsky Reserve (Russian Far East; Kamchatka). Eight litters of foxes were observed during June - September 2013, 2014, 2015, 2016. Each family of foxes we monitored with 3 - 6 photo-traps installed on their den territory. 228912 shots were processed, of which 1108 recorded older individuals feed the cubs (milk and solid food). We confirmed feed description by the analysis of excrements, which were collected every three days from each den site; 50 samples were examined. Data base included – date, time, frame number, den site, identified adult feeder individual, type and amount of food. All moments when puppies were fed with milk were counted.

The volume of food got by an adult fox during feeding puppies lesser depends on the density of food objects, and more on the number of cubs in the litter. Voles ( $89 \pm 7\%$ ) are the basis of the diet of foxes both in Tundra and Ocean coast biotopes. The number of voles for each cub is about  $120 \pm 14$  voles (before self-feeding age), not depending on the litter size and on the number of adults who feed the cubs. There were a bird (2 – 14 %), fish (1 – 2 %) and Beringian ground squirrel (1 – 3 %) in the fox cub diet. Foxes also use berries; sprouts of cedar pine nuts; insects. Using of food types is seasonal (except for voles). At the cubs' age of 5 - 6 weeks adults stop feeding them with milk. Intervals between feedings are variable (from  $\frac{3}{4}$  to 6 hours). The number of voles per day range from 0 to 90. The maximum of plant food and insects used coincides with the transition of the cubs to an independent way of feeding (8 - 9 weeks). There are two strategies for distributing parental efforts for fox families: [1] to bring more food with rare appearing on the den site; [2] bring less food but often appear on the den site. Distribution of duties corresponds to social roles in a fox family. In family without male participation, two females cope with the feeding of the younglings and distributed roles similarly. Thus in families of any composition two individuals have almost equal importance.

Financially supported by Kronotsky Nature Reserve; RFBR grant #13-04-00192a, MAB UNESCO Young Scientists Award.

## Appearance of play behaviours during development of fox cubs

YACHMENNKOVA ANNA A<sup>1,2</sup>, KORENKOVA ANNA A<sup>3\*</sup>

<sup>1</sup>Severtsov Institute of Ecology and Evolution, Russian Academy of Science, Leninsky pr. 33, Moscow, 117071, RUSSIA

<sup>2</sup>Kronotsky Nature Reserve, Ryabikova Street, 48, Yelizovo, Kamchatka region, 684000, RUSSIA

<sup>3</sup>Lomonosov Moscow State University, GSP1, Leninskie Gory, Moscow, 119991, RUSSIA; korenkovaa@mail.ru

Play is one of the most common processes in the development of animal behaviour. Play is extremely important for the development of behavioural norms for all mammals. We observed fox cubs play behaviour development at the age of 3 - 13 weeks in the wild during June - August 2013, 2014, 2015 on the territory of Kronotsky Natural Reserve, MAB UNESCO, Kamchatka, Russia. We collected data by using camera traps, which we established nearby fox dens. Also we collected video episodes of fox day activity. We analysed videos of plays between sib-pairs(40,7 min) "frame to frame" by using Observer XT (Noldus). Most of the behaviour elements appear in play at 4<sup>th</sup> week, appearing of new elements stops at the age of 8,5 weeks. Mousing appears in play after parents bring to pups first rodents to feed them; this happens before pups start trying to catch insects or voles. At first (5<sup>th</sup> week), cubs mouse without aim, next week they start mousing at subjects. Thus, simple elements can complete a base for new and more elements. Digging elements appear after parent's demonstration of this type of behaviour to cubs, and before cubs begin to dig den-holes. In similar age (6<sup>th</sup> week) mounting each other appears; together with digging it is included the reproductive behaviour. Cubs express new behavioural elements by integrating them into their plays. Different elements that belong to the same behavioural complex appear together. In addition, 16 main play elements are expressed throughout development. Plays become more complex when cubs grow up; there is an increase in – [1] the number of different elements expressed, [2] the frequency of elements that follow each other, [3] the completeness of elements, and [4] duration of play. Totally we described 4 independent age periods of fox play behaviour development: I – unformed play (at the age of 3 weeks); II – the appearance of the full play sequence (at the age of 4 weeks); III - training-period on the social partners (at the age of 5 - 8 weeks), and IV – reducing the role of play activity (at the age of 9 - 13 weeks). All of these periods are associated with stages of development of cubs' self-sufficiency, what influence to cubs' social preferences in plays. We proposed a special scale-scheme of fox cubs play behaviour ontogenesis which is a system of data on cub development by organizing and lining them up chronologically. Financially supported by Kronotsky State Nature Reserve and RFBR grant #13-04-00192a.

## **Selected endocrine mechanisms regulating the reproductive processes of the European Roe deer (*Capreolus capreolus*)**

ZADROGA ANNA, GÓRZYŃSKA IZABELA, KORZEKWA ANNA

Department of Biodiversity Protection, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima St. 10, 10-747 Olsztyn, POLAND; a.zadroga@pan.olsztyn.pl

Pregnancy in European roe deer varies in length which could be dependent on the time of fertilisation. Pregnancy prolongation was observed during the implantation process in the roe deer uterus (embryonic diapause). To our knowledge, no data exist concerning molecular factors involved in the regulation of reproductive processes in the oestrous cycle, anoestrous and pregnancy including embryonic diapause in European roe deer. Progesterone (P4) is a steroid hormone that is mainly produced during pregnancy; by luteal cells of corpus luteum in the luteal phase and early pregnancy and is synthesized by placenta during later pregnancy. Pregnancy-associated glycoproteins (PAGs) are specific for the placental trophoblast indicate the end of implantation after the diapause. The number of roe deer populations gradually increase and the increasing number of individual animals could depend on environmental and ecosystem changes which are of more extreme in last fifty years. The knowledge concerning reproduction of this species might help to understand how quantity is regulated in a sustainable ecological way. Concerning those aspects, the aim of our study was to determine if the P4 synthesis pathway in roe deer reproductive processes and PAGs expression can be used as markers to either confirm or exclude diapause in examined individuals. Therefore uterus` and peripheral blood gene expression of pregnant and non-pregnant roe deer females (from North-East of Poland) were measured for PAGs, P4 receptor (P4RA) and the enzymes: 3beta-hydroxysteroid dehydrogenase (3BHSD) and 20alpha-hydroxysteroid dehydrogenase (20AHSD) was measured by Real-Time PCR. Protein concentrations of 3BHSD and, 20AHSD, both subunits of P4 receptor, were determined by Western blotting. The level of PAGs and P4 protein concentration in blood plasma were measured by enzyme-linked immunosorbent assay (ELISA).

The gene expression of enzymes involved in P4 synthesis were significantly higher in pregnant females compared to non-pregnant ( $p < 0.05$ ), even though we did not detect differences between proteins concentration ( $p > 0.05$ ). The level of P4 subunits protein expression remained unchanged ( $p > 0.05$ ). However, P4 protein concentration in blood plasma was higher in pregnant than in non-pregnant individuals ( $p < 0.05$ ). PAGs mRNAs and proteins were undetectable.

Our results indicate that P4 is involved in maintaining pregnancy in roe deer. Results concerning the lack of PAGs on mRNA and protein level suggest that diapause occur in most investigated roe deer individuals. Further studies are necessary to find molecular mechanism of diapause in European roe deer.

## **A balancing act – saving gametes of aged felids**

ZAHMEL JENNIFER, MÜLLER KARIN, FERNANDEZ LORENA, JEWGENOW KATARINA

Leibniz-Institute for Zoo and Wildlife Research, Dept. Reproduction Biology, Alfred-Kowalke-Str. 17, 16230, Berlin, GERMANY; zahmel@izw-berlin.de

Regarding the rapid loss of biodiversity, storage of frozen biomaterials is an important tool to preserve the genetic information and variety of endangered species. Within this scenario, zoos are not only predestined to contribute to biobanking by providing samples, they also commit themselves to sustain or re-establish captive living animal populations by professional management of breeding programs. Since 2007, the Leibniz-Institute for Zoo and Wildlife Research has been running a felid gamete bank consisting of oocytes, sperm cells, embryos, ovarian and testicular tissue for (i) preserving genes of important animals to bring them back into the captive populations and (ii) developing the methods of assisted reproductive techniques (ART), like artificial insemination (AI), *in vitro* maturation (IVM) and fertilisation of oocytes (IVF), and embryo transfer. Samples were provided by zoos mostly after euthanasia due to age or health issues.

Gonads were shipped to the IZW per overnight express. Gametes were extracted from ovaries and epididymides, and assessed for quality. Sperm cells were cryopreserved if possible. Oocytes were subjected to *in vitro* maturation, and if possible were fertilised and checked for embryonal cleavage. In a few cases, oocytes were cryopreserved directly. Embryos with promising developmental potential were cryopreserved for future embryo transfer studies.

Gonads of 76 female and 67 male felids and 19 species were offered to the bank. 30 % of them were at least 14 years old. In 30.3 % of all samples no gametes were isolated. Sperm cells of 36 individuals (12 species, including two tiger and four leopard subspecies) were cryopreserved. At least 61 % of cryopreserved samples can be used exclusively for *in vitro* fertilisation and 7/36 samples can be only used when performing intracytoplasmic sperm cell injection due to poor quality. In total, 1072 oocytes were isolated. 278 oocytes reached a fertilisable maturation stage *in vitro* and 43 embryos have been produced from 5 different species.

Although sample availability is limited, shipment of samples is needed, and many samples originated from aged animals with unknown health issues, a remarkable number of samples could be processed and resulted in embryo production or preserved gametes. There is still a big potential in the improvement of methodologies but this in turn demands gamete samples. Zoos are strongly encouraged to support gamete banking but should be aware of the fact, that gamete quality is age-related and AI capable sperm quality cannot be expected in overaged individuals.

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