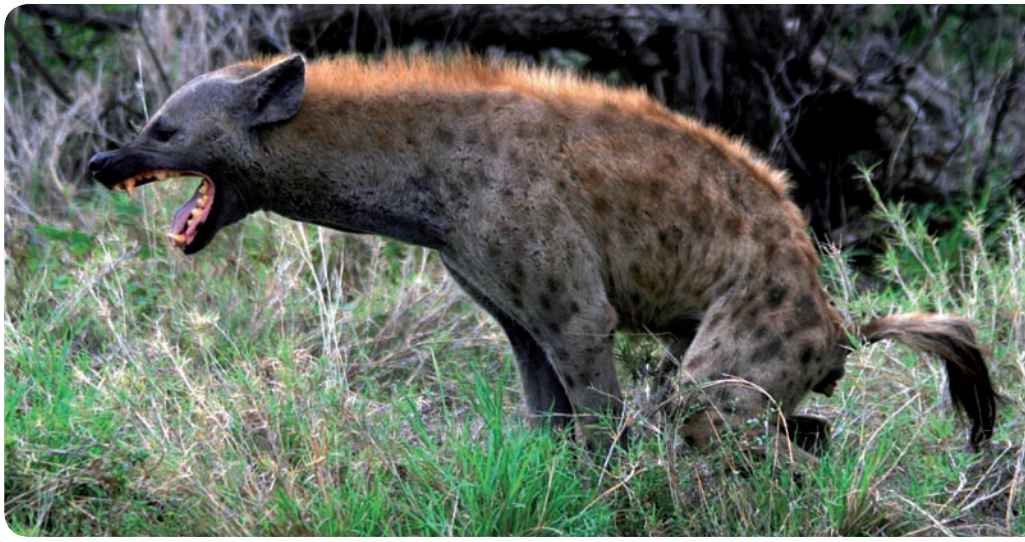
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Proceedings of the Conference on

"Non-invasive Monitoring of Hormones"

(3rd annual ISWE meeting)

Vetmeduni Vienna, Austria

September 23rd to 26th 2012

Edited by:
Rettenbacher, S.,
Vick, M. and Palme, R.

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Titelbild (Title picture): defecating spotted hyena (© Dr. Gerd Wähner)

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Editorial

Welcome to the conference

"Non-invasive Monitoring of Hormones"

(**3rd annual meeting** of the International Society of Wildlife Endocrinology)

Over the past decades, non-invasive monitoring of endocrine activity via faeces, urine or saliva has become an important tool not only for reproductive management but also for the investigation of questions in the fields of animal welfare, animal husbandry, ecology and conservation biology. Furthermore, application of the method has opened new perspectives on biomedical and behavioural sciences.

The former Institute of Biochemistry (now part of the Institute of Medical Biochemistry) of the University of Veterinary Medicine in Vienna has a long tradition in faecal steroid analysis. Exactly 30 years after introducing the first assay for measuring oestrogens in faecal samples of cows for pregnancy diagnosis and 15 years after the first description of a specifically designed enzyme immunoassay for measuring a group of faecal cortisol metabolites, it is now our great pleasure to host a conference on these methods.

The aim of this conference is to bring together scientists of different research fields who share a common interest in non-invasive methods for monitoring hormones. We hope that this meeting will provide an excellent opportunity to learn about newest developments and successful applications, to come in contact with experts in the field and to meet old and new collaborators and friends.

The majority of the over one hundred contributions focus on the non-invasive assessment of **Reproduction** and **Stress**, as these topics have received most attention in the past. **Methodological issues** are challenging and demanding and we have decided to emphasize their importance by dedicating a session to them. Finally, the sections **New avenues** and **Miscellaneous topics** should enable participants to become familiar with the newest approaches and emerging hot topics in the field and we hope they will stimulate new directions of research and applications. Each session will be opened by two invited keynote lectures, followed by selected talks and completed by several poster presentations on the same topic.

Thus, we look forward to fruitful and stimulating discussions in the cosy atmosphere of our University campus. Besides an interesting conference, we also hope that you will enjoy Vienna's unique charm and special flair, a metropolis of culture, music and arts.

On behalf of the local organizing committee we warmly welcome you in Vienna!

Sophie Rettenbacher (Vetmeduni Vienna)
Mandi Vick (ISWE)
Rupert Palme (Vetmeduni Vienna)

Acknowledgements

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Meredith J. Bashaw (Franklin & Marshall College, Lancaster, PA, USA)

Kerry Fanson (Deakin University, Geelong, Australia)

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Marina Ponzio (CONICET, University of Córdoba, Argentina)

Rachel Santymire (Lincoln Park Zoo, Chicago, IL, USA)

Catharine Wheaton (Disney's Animal Kingdom, Bay Lake, FL, USA)

Sue Walker (Chester Zoo, Upton-by-Chester, UK)

Mandi Vick (Cleveland Metroparks Zoo, Cleveland, OH, USA)

We warmly thank all our invited speakers for contributing keynote lectures:

Janine Brown, Martin Dehnhard, Tobias Deschner, Tim Ellis, Michael Heistermann, Katharina Hirschenhauser, Erich Möstl, Chadi Touma, Nadja Wielebnowski

Technical Support:
Michael Lepschy, Mareike Stöwe

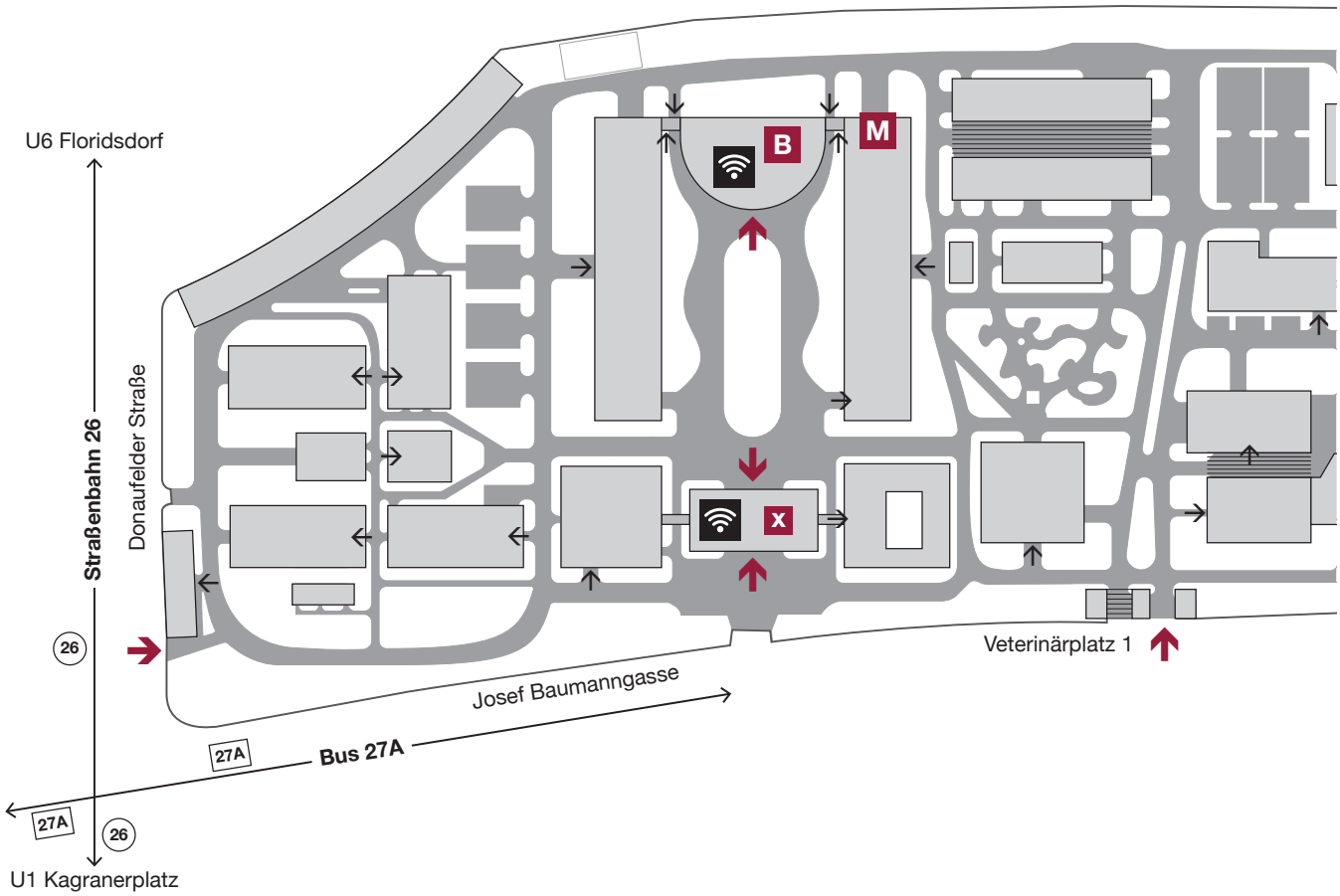
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Map of Campus



Important Information

Venue
University of Veterinary Medicine Vienna
"Vetmeduni Vienna"
Veterinärplatz 1, 1210 Vienna, Austria

Contact Conference Manager
Austropa Interconvention
Ms. Sandrina Sinko
Phone: +43/1/588 00-511

Welcome Reception/Get-Together/Poster Setup
Sunday, 23 September
6 p.m. - 9 p.m.
Snacks and drinks will be served in the Aula of the University (see "x" on the map)

Registration Opening Times

Sunday, 23 September
6 p.m. - 8 p.m.
Monday, 24 September
8:30 - lunch time
Registration desk will be in the Aula (Sunday) or in front of lecture hall B (Monday).

Poster Area

Posters will be exhibited in the hall in front and the corridors around lecture hall B.

Coffee Breaks / Lunch

Coffee will be served in front of lecture hall B.
Lunch will be served in the Aula.

Poster Party

Monday, 24 September
6 p.m. - 9 p.m.
Snacks and drinks will be served in the hall in front and the corridors around lecture hall B.

Conference Dinner

Tuesday, 25 September
Dinner at Heuriger Wolff
Rathstraße 50 in Neustift am Walde, 1190 Wien
Busses will leave from the Vetmeduni (main gate) at 7 p.m.
Busses will return to Vetmeduni at about 11 p.m.

Excursion

Wednesday, 26 September
Visit of the Vienna Zoo Schönbrunn
Underground line U4 direction Hütteldorf – Station Hietzing (Zoo symbol)
Walking distance from underground to Hietzinger entrance of the zoo: 10 min.
Meeting point is 2:30 p.m. at the entrance gate of the Zoo

Internet Access

Free WLAN is available for conference participants on two locations (see map).
User Name: endocrinology
Password: SWmHC4qJ
SSID: Veranstaltungen

How to connect:
1. activate WLAN-Search on your device
2. connect with “Veranstaltungen“
3. open your webbrowser and choose any webside
4. first time you will be automatically redirected to a login-page where you have to enter the above provided data.
5. after logging in successfully internet over WLAN is available during the whole conference

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INTERNATIONAL SOCIETY of WILDLIFE ENDOCRINOLOGY

vetmeduni vienna

Programme ISWE 2012
"Non-invasive Monitoring of Hormones"
23. – 26. Sept 2012, Vetmeduni, Vienna

Sunday, 23.9.2012

09:00-15:30: ISWE Board meeting
15:30-17:30: ISWE membership general session and voting (lecture hall M)
18:00-21:00: Registration, welcome reception, informal get-together (poster setup)

Monday, 24.9.2012

8:30-9:00: Registration
9:00-9:30: Opening address

Reproduction

(chair: Linda Penfold, Yulee, US)

Keynote lectures

9:30-10:15: Janine Brown (Front Royal, US): Hormone monitoring of wildlife species: An important tool for studying reproduction and aiding breeding management
10:15-11:00: Michael Heistermann (Göttingen, Germany): Non-invasive assessment of physiological status: A 30-year contribution to primatology

11:00-11:30: Coffee Break

Selected free talks (11:30-12:45)

Catherine Wheaton	Tolerance may lead to loss of tenure in tamarin societies: Use of non-invasive faecal reproductive steroid monitoring and behaviour observations to reveal reproductive and life-history traits in wild female cotton-top tamarins (*Saguinus oedipus*) in Colombia
Tamara Keeley	The evaluation of reproductive hormones to determine the efficacy of commercial GnRF vaccines as effective contraceptive options in captive female Asian elephants
Tatiana Micheletti	The use of altrenogest to reduce ovarian activity and avoid hyperoestrogenism after eCG-hCG ovulation protocol in tigrina (*Leopardus tigrinus*)
Ekaterina Pavlova	Seasonal hormonal adaptations in felid reproduction
Jocelyn Bryant	Faecal hormone metabolite monitoring of reproductive patterns and seasonality in red river hogs (*Potamochoerus porcus*)

Lunch break until 14:00

Methodological issues

(chair: Janine Brown, Front Royal, US)

Keynote lectures

14:00-14:45: Erich Möstl (Vienna, Austria): Remnants of stress hormones: Measurement and biological activity
14:45-15:30: Tobias Deschner (Leipzig, Germany): LC-MS analyses of steroid hormones or their metabolites from faeces and urine: Problems and perspectives

15:30-16:00: Coffee Break

Selected free talks (16:00-17:30)

Michael Lepschy	Steroid extraction: Get the best out of faecal samples
Katie Edwards	A practical field extraction method for non-invasive monitoring of hormonal activity in the black rhinoceros, *Diceros bicornis*
Otto Kalliokoski	The confounding effect of GI activity on reliability of faecal glucocorticoids as biomarkers of stress
Sophie Rettenbacher	Is stress of the female bird reflected in her eggs?
Juliane Riechert	Multiple sampling of common terns via blood-sucking bugs: Change of prolactin and corticosterone values during incubation
Andy Kouba	A novel method for non-invasively determining identity, gender, and reproductive status in vertebrates using Faecal Near Infrared Reflectance Spectroscopy (FNIRS): Case study results from okapi and giant panda.

18:00-21:00: Poster Party

Tuesday, 25.9.2012

Stress

(chair: Andre Ganswindt, Pretoria, South Africa)

Keynote lectures

9:00-9:45: Nadja Wielebnowski (Portland, US): The role of faecal glucocorticoid metabolite monitoring for welfare assessments and conservation management of wildlife

9:45-10:30: Chadi Touma (Munich, Germany): Non-invasive monitoring of stress hormones in rodents and its application in biomedical research

10:30-11:00: Coffee Break

Selected free talks (11:00-12:30)

Martin Amrein	The effect of a fission-fusion zoo housing system on hormonal indicators of stress in Bornean orangutans (*Pongo pygmaeus*)
Marina Munerato	Hormonal response to capture stress in free-ranging pampas deer (*Ozotoceros bezoarticus*)
Lara Metrione	Living with the threat of predation: Faecal corticosterone metabolites in bison
Carole Fureix	Do stereotypic horses cope better with poor environmental conditions? A physiological approach
Jim Clapp	Quantifying fearfulness and aggression in beef cattle using saliva hormone measurements
Lisa Maria Glenk	Can dogs relax? Work-related cortisol levels vary in dogs during animal-assisted interventions: A pilot study

Lunch break until 14:00

New avenues

(chair: Jonathan Aaltonen, Omaha, US)

Keynote lectures

14:00-14:45: Martin Dehnhard (Berlin, Germany): Non-invasive pregnancy diagnosis in felids based on faecal prostaglandin F2α metabolites

14:45-15:30: Tim Ellis (Weymouth, England): Non-invasive monitoring of hormones in fish

15:30-16:00: Coffee Break

Selected free talks (16:00-17:00)

Luana Cortinovis	Preliminary study on non-invasive methods for hormonal profile determination in the basking shark (*Cetorhinus maximus*)
David Kersey	Validating thyroxine and triiodothyroxine assays for use in measuring thyroid hormone metabolites excreted in the faeces and urine of the female giant panda.
Cedric Girard-Buttoz	Practicalities of urinary c-peptide measurements for monitoring the nutritional status of wild animals
Liza Moscovice	Peripheral oxytocin levels in wild female chacma baboons fluctuate with oestrous state and involvement in sexual consortships

Evening: Conference dinner at Heuriger Wolff

Wednesday, 26.9.2012

Miscellaneous topics

(chair: Tamara Keeley, Dubbo, Australia)

Keynote lecture

9:00-9:45: Katharina Hirschenhauser (Seewiesen, Germany): Excreted steroid metabolites in bird droppings: Behavioural studies using baseline patterns and stimulus-specific responses

Selected free talks

9:45-10:30:

Mareike Stöwe	Social context: A modulator of behaviour and glucocorticoid secretion
Sascha Rösner	Wildlife management of a highly endangered species: The capercaillie (*Tetrao urogallus*) – implications from behavioural and physiological indicators
Rachel Santymire	Difficulties of determining pregnancy non-invasively in the endangered black-footed ferret

10:30-11:00: Coffee Break

Selected free talks (11:00-12:30)

Antje Engelhardt	Female signals of fertility and male reproductive behaviour in macaques
Bernard Wallner	Macaque mothers' pre-conception faecal testosterone levels relate to dominance and to sex of offspring
Andre Ganswindt	Endocrine drivers of flexible rutting behaviour in male giraffes (*Giraffa camelopardalis*)
Jennifer Sanderson	Carers and non-carers respond differently to a territorial challenge; evidence for a physiological division of labour in the banded mongoose (*Mungos mungo*)
Verena Behringer	Adrenarche in bonobos (*Pan paniscus*): Evidence from ontogenetic changes in urinary dehydroepiandrosterone-sulfate (DHEA-S) levels
Mathias Nemeth	Saliva cortisol in relation to cognition and behaviour after PUFA administration in guinea pigs

12:30: Closing remarks - official end of the conference

14:30: Visit of the Vienna Zoo Schönbrunn – free entrance – guided tours

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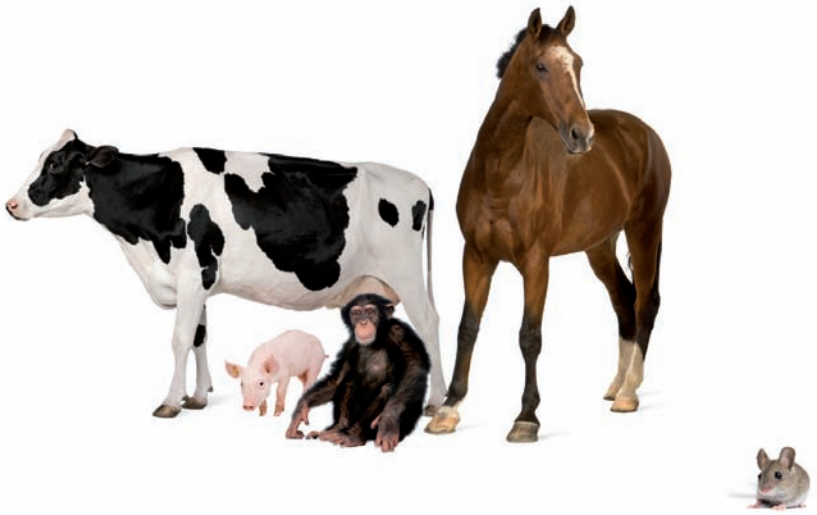
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List of Presentations

Talks

Reproduction (TR 1-7)

1.	Janine Brown	Hormone monitoring of wildlife species: An important tool for studying reproduction and aiding breeding management (keynote lecture)
2.	Michael Heistermann	Non-invasive assessment of physiological status: A 30-year contribution to primatology (keynote lecture)
3.	Catherine Wheaton	Tolerance may lead to loss of tenure in tamarin societies: Use of non-invasive faecal reproductive steroid monitoring and behaviour observations to reveal reproductive and life-history traits in wild female cotton-top tamarins (*Saguinus oedipus*) in Colombia
4.	Tamara Keeley	The evaluation of reproductive hormones to determine the efficacy of commercial GnRF vaccines as effective contraceptive options in captive female Asian elephants
5.	Tatiana Micheletti	The use of altrenogest to reduce ovarian activity and avoid hyperoestrogenism after eCG-hCG ovulation protocol in tigrina (*Leopardus tigrinus*)
6.	Ekaterina Pavlova	Seasonal hormonal adaptations in felid reproduction
7.	Jocelyn Bryant	Faecal hormone metabolite monitoring of reproductive patterns and seasonality in red river hogs (*Potamochoerus porcus*)

Methodological issues (TMe 1-8)

1.	Erich Möstl	Remnants of stress hormones: Measurement and biological activity (keynote lecture)
2.	Tobias Deschner	LC-MS analyses of steroid hormones or their metabolites from faeces and urine: Problems and perspectives (keynote lecture)
3.	Michael Lepschy	Steroid extraction: Get the best out of faecal samples
4.	Katie Edwards	A practical field extraction method for non-invasive monitoring of hormonal activity in the black rhinoceros, *Diceros bicornis*
5.	Otto Kalliokoski	The confounding effect of GI activity on reliability of faecal glucocorticoids as biomarkers of stress
6.	Sophie Rettenbacher	Is stress of the female bird reflected in her eggs?
7.	Juliane Riechert	Multiple sampling of common terns via blood-sucking bugs: Change of prolactin and corticosterone values during incubation
8.	Andy Kouba	A novel method for non-invasively determining identity, gender, and reproductive status in vertebrates using Faecal Near Infrared Reflectance Spectroscopy (FNIRS): Case study results from okapi and giant panda

Stress (TS 1-8)

1.	Nadja Wielebnowski	The role of faecal glucocorticoid metabolite monitoring for welfare assessments and conservation management of wildlife (keynote lecture)
2.	Chadi Touma	Non-invasive monitoring of stress hormones in rodents and its application in biomedical research (keynote lecture)
3.	Martin Amrein	The effect of a fission-fusion zoo housing system on hormonal indicators of stress in Bornean orangutans (*Pongo pygmaeus*)
4.	Marina Munerato	Hormonal response to capture stress in free-ranging pampas deer (*Ozotoceros bezoarticus*)
5.	Lara Metrione	Living with the threat of predation: Faecal corticosterone metabolites in bison
6.	Carole Fureix	Do stereotypic horses cope better with poor environmental conditions? A physiological approach
7.	Jim Clapp	Quantifying fearfulness and aggression in beef cattle using saliva hormone measurements
8.	Lisa Maria Glenk	Can dogs relax? Work-related cortisol levels vary in dogs during animal-assisted interventions: A pilot study

New avenues (TNa 1-6)

1.	Martin Dehnhard	Non-invasive pregnancy diagnosis in felids based on faecal prostaglandin F2α metabolites (keynote lecture)
2.	Tim Ellis	Non-invasive monitoring of hormones in fish (keynote lecture)
3.	Luana Cortinovis	Preliminary study on non-invasive methods for hormonal profile determination in the basking shark (*Cetorhinus maximus*)
4.	David Kersey	Validating thyroxine and triiodothyroxine assays for use in measuring thyroid hormone metabolites excreted in the faeces and urine of the female giant panda
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6.	Liza Moscovice	Peripheral oxytocin levels in wild female chacma baboons fluctuate with oestrous state and involvement in sexual consortships

Miscellaneous topics (TMi 1-10)

1.	Katharina Hirschenhauser	Excreted steroid metabolites in bird droppings: Behavioural studies using baseline patterns and stimulus-specific responses (keynote lecture)
2.	Mareike Stöwe	Social context: A modulator of behaviour and glucocorticoid secretion
3.	Sascha Rösner	Wildlife management of a highly endangered species: The capercaillie (*Tetrao urogallus*) – implications from behavioural and physiological indicators
4.	Rachel Santymire	Difficulties of determining pregnancy non-invasively in the endangered black-footed ferret
5.	Antje Engelhardt	Female signals of fertility and male reproductive behaviour in macaques
6.	Bernard Wallner	Macaque mothers' pre-conception faecal testosterone levels relate to dominance and to sex of offspring
7.	Andre Ganswindt	Endocrine drivers of flexible rutting behaviour in male giraffes (*Giraffa camelopardalis*)
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9.	Verena Behringer	Adrenarche in bonobos (*Pan paniscus*): Evidence from ontogenetic changes in urinary dehydroepiandrosterone-sulfate (DHEA-S) levels
10.	Mathias Nemeth	Saliva cortisol in relation to cognition and behaviour after PUFA administration in guinea pigs

Posters

Reproduction (PR 1-23)

1.	Annemieke van der Groot	Faecal progestagen patterns in wild African white rhinoceros (*Ceratotherium simum*)
2.	Franz Schwarzenberger	Reproductive steroid monitoring in white rhinoceroses kept in European zoos
3.	Katie Edwards	Using hormone analysis to investigate reproductive success in the female eastern black rhinoceros (*Diceros bicornis michaeli*)
4.	Jess Trotter	Relationship between management, adrenal activity and reproduction in a captive group of female Asian elephants (*Elephas maximus*)
5.	Ulrike Pfeiffenberger	Faecal steroid monitoring as a supportive tool for the reproductive management of okapis kept in European zoos
6.	Susan Walker	Reproductive cyclicity in female okapi (*Okapia johnstoni*): The potential role of mate compatability?
7.	Katherine Cho	Pregnancy diagnosis in wild equids, the Przewalski's horse (*Equus caballus przewalskii*) and onager (*Equus hemionus onager*)
8.	Evelina Zanetti	Comparison of two different superovulation protocols on ovarian and adrenal activity in the brown brocket deer (*Mazama gouazoubira*)
9.	Priscila Viau	Analytic profile of oestrogens and progestins in different biological matrixes in the ovine (*Ovis aries*)
10.	Katherine MacKinnon	Assessment of the reproductive physiology of the potto (*Perodicticus potto*)
11.	Barbara Tiddi	Influence of the ovarian cycle stage on female proceptive behaviours and male mating activity in wild tufted capuchin monkeys
12.	Marcelo Guimarães	Non-invasive monitoring of reproductive endocrine profile and socio-sexual behaviour in captive black-tufted marmoset (*Callithirx penicillata*)

13.	Monique Paris	Reproductive physiology of the world's rarest canid: Patterns in oestradiol, progesterone, glucocorticoid and testosterone metabolites in Ethiopian wolves (*Canis simensis*)
14.	Itsuki Adachi	Pregnancy detection and endocrinological regulation of parturition in Tsushima leopard cat (*Prionailurus bengalensis euptilurus*)
15.	Johanna Painer	Reproductive hormones in serum samples from free ranging Eurasian lynx (*Lynx lynx*) and their value for reproductive assessment
16.	Letitia Zezza	Non-invasive monitoring of sexual profile in two pubertal captive polar bears (*Ursus maritimus*)
17.	Karen Steinman	Validation of enzyme immunoassays for the measurement of reproductive hormones in polar bear (*Ursus maritimus*) urine
18.	Mandi Vick	Non-invasive monitoring of reproductive hormones in faecal samples from sloth (*Melursus ursinus*) and Andean (*Tremarctos ornatus*) bears, preliminary findings
19.	Marie-Odile Chelini	Adrenal glands release oestrogens in response to an ACTH challenge: A possible mechanism for stress induced reproductive dysfunction
20.	Marina Ponzio	Evaluation of three immunoassay systems for the assessment of urinary biomarkers of ovarian function in the endangered chinchilla (*Chinchilla lanigera*)
21.	Eva Millesi	Ovarian activity in captive and free-ranging European ground squirrels during the breeding and post-breeding period
22.	Annalisa Zaccaroni	Non-invasive methods for the hormonal profile determination in eels
23.	Constanza Formigaro	Comparison of hormonal screening results in placenta tissue and blood of orca (*Orcinus orca*)

Stress (PS 1-35)

1.	Diana Armstrong	Ape behaviour and hormonal response to alternation between zoo enclosures at Lincoln Park Zoo
2.	Christof Neumann	Stress levels, dominance rank and hierarchy dynamics in wild male crested macaques (*Macaca nigra*)
3.	Assahad Gholib	Number of fertile females and male social status influence physiological stress levels in wild male crested macaques (*Macaca nigra*)
4.	Carlo Cinque	Relocation stress induced long-term faecal hormonal modifications in semi free-ranging social groups of *Macaca tonkeana*
5.	Brandon Wheeler	Feeding competition and stress in wild tufted capuchin monkeys: Implications for the evolution of "deceptive" alarm calling
6.	Leticia Martinez	Comparison of faecal cortisol metabolite concentrations in white rhinoceroses (*Ceratotherium simum*) living in three different environments
7.	Sara Cáceres	Faecal cortisol and progesterone metabolites concentration in four captive white rhinoceros (*Ceratotherium simum*) kept in different housing conditions
8.	Julia Eggermann	Human activity is not the main source of stress in wolves
9.	Leanne van der Weyde	Adrenal activity in captive and free-ranging African wild dogs (*Lycaon pictus*) during the breeding season
10.	Bart Vlamings	Dog appeasing pheromone®: A useful tool to minimize stress and aggression in African wild dogs (*Lycaon pictus*)?
11.	Nadja Kneidinger	The use of non-invasive monitoring methods in conservation breeding of the endangered European mink (*Mustela lutreola*)
12.	Kerry Fanson	Individual stress response predicts post-release survival in reintroduced Canada lynx
13.	Maik Rehnus	Influence of tourism activities on glucocorticoid secretion in mountain hares (*Lepus timidus*)
14.	Elizabeth Freeman	Evaluation of faecal glucocorticoid metabolites in captive red pandas (*Ailurus fulgens*)
15.	Robyn Pimm	Response of female veiled chameleons (*Chameleo calyptratus*) in a research colony to environmental disturbances
16.	Juan Busso	Assessment of food-based enrichment in collared anteater (*Tamandua tetradactyla*) by non-invasive monitoring of adrenocortical activity
17.	Michaela Brenner	Anthropogenic effects on European ground squirrels physiology
18.	Carina Siutz	Body fat content and faecal cortisol secretion patterns in free-ranging juvenile common hamsters

19. Katie Graham	Preliminary investigation of the use of non-invasive measurements of faecal immuno-reactive corticosterone in rufous fantails (*Rhipidura rufifrons*): A potential diagnostic tool for choice of best candidates for translocation
20. Alvaro Navarro-Castilla	Motorways as stressors for wild wood mouse (*Apodemus sylvaticus*) populations
21. Vera Voznessenskaya	Perception of sex pheromones in closely related mus species is modulated by glucocorticoids
22. Joao Ferreira	Non-invasive measurement of adrenocortical activity in blue-fronted parrot (*Amazona aestiva*)
23. Nicoletta Formenti	Evaluation of the physiological stress response induced by winter sports in a black grouse (*Tetrao tetrix*) population from Lepontine Alps (VB)
24. Sonja Ludwig	Effects of mate separation on stress hormones, parasite load and immune parameters in free-living greylag geese
25. Michaela Spreafico	Physiological and behavioural response in carrion crows (*Corvus corone corone*) after relocation to a new environment
26. Georgine Szipl	Vocal response to stressful situations in carrion crows (*Corvus corone corone*)
27. Matthias Loretto	Does coping style affect preferences for feeding sites in ravens?
28. Witold Kędzierski	Plasma and salivary cortisol levels as the indicators of stress and fatigue during field exercise test in thoroughbred horses
29. Line Ahrendt	Relationship between social rank and adrenocortical activity in horses
30. Dean Konjević	Non-invasive monitoring of adrenocortical activity in captive fallow deer (*Dama dama L*)
31. Eva Van laer	Effect of mild heat stress on faecal cortisol metabolites, blood minerals and energy metabolism of Holstein cows
32. Holly Boland	Determination of stress in beef calves weaned using different methods
33. Leire Ruiz	Non-invasive measures of acute stress reactivity in European rabbit (*Oryctolagus cuniculus L.*) restocking fences and their relation to fitness
34. Ramón Rivera	Using non-invasive methods to determine animal stress in surgical training models

Methodological issues, New avenues & Miscellaneous topics (PM 1-10)

1. Agnes Haymerle	Faecal cortisol metabolites to assess stress in wildlife: Evaluation of a field method on free ranging chamois
2. Rebecca Rimbach	Validation of a faecal glucocorticoid assay and effects of daytime, sex and female reproductive state on glucocorticoid output in spider monkeys (*Ateles hybridus*)
3. Mohammad Agil	Biological validation of enzyme immunoassays for measurement of faecal androgen and glucocorticoid metabolites in crested macaques (*Macaca nigra*)
4. Stefanie Petow	A new quantification method for the analysis of non-metabolized faecal cortisol with a commercial ELISA Kit
5. Meredith Bashaw	Assay development for pregnancy testing of sitatunga (*Tragelaphus spekei*)
6. Esther Carlitz	Monitoring stress in captive and wild orangutans with cortisol measurements obtained from shed nest hair?
7. Linda Hofman	Levels of cortisol in hair samples of non-human primates
8. Russ Hart	A preliminary investigation for monitoring reproductive status using prostaglandin F2-alpha metabolite (PGFM) and proposed pregnancy biomarker ceruloplasmin in the urine of giant pandas
9. Irene de la Casa	Endocrine disruption associated to oral administration of atrazine in European quail (*Coturnix coturnix coturnix)*
10. Myrna Campos Ferraz	Effect of brute propolis on the diet of *Callithrix jacchus* used in research: Maintenance of the body weight







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Abstracts

TR1 – keynote lecture

Hormone monitoring of wildlife species: An important tool for studying reproduction and aiding breeding management

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As more species reach vulnerable or endangered status, zoos are increasingly being tasked with sustaining genetically healthy populations in case of catastrophic extinctions. Unfortunately, many captive populations are not self-sustaining. It is now clear that the ability to track reproductive activity via hormones is key to developing successful *ex situ* breeding programmes, through both natural and assisted reproductive means. What we have observed is a diversity of reproductive mechanisms used among the various taxa, even within closely related species. For example, among the *Felidae*, females of all species are induced ovulators, but in some species, females also exhibit spontaneous ovulations, and this can vary between individuals within a species. There are marked species differences in the impact of season on reproductive function, with some being exquisitely sensitive to photoperiod (e.g. Pallas' cat), some moderately affected (tiger, clouded leopard, snow leopard), and others that are not influenced at all (e.g. ocelot, tigrina, margay, lion, leopard, fishing cat). One of the biggest challenges in *ex situ* breeding management is overcoming problems associated with highly variable ovarian responses to ovulation induction therapies used with assisted reproductive procedures, like artificial insemination (AI). Success is relatively high in the cheetah and ocelot, but few pregnancies have resulted after AI in clouded leopard, fishing cat and tiger. Among *Perrisodactyla*, there is a wide range in cycle lengths, including the finding that some species exhibit cycles of differing lengths (observed in white rhino and Malayan tapir). Presented are examples of how hormone monitoring has been used to study reproduction and enhance captive management efforts of diverse species. Many of these findings have been incorporated into husbandry manuals for specific species. Important endocrine findings related to animal management include: 1) learning that cheetah females experience reproductive suppression if housed together; 2) finding that reproduction in the seasonal Pallas' cat is negatively affected by altered photoperiod (e.g., a zoo's festival of lights); 3) the discovery of ovarian acyclicity in elephants and its relationship to socio-environmental factors; and 4) reinforcing how reproductive monitoring can aid breeding introductions of stress-susceptible species – canids, felids, many ungulates, even some primates. Given the relative ease of sample collection for most species (i.e., faeces or urine) and critical need to increase birth rates within captive populations, it is imperative to establish hormone monitoring programmes to aid basic research and applied management efforts.

TR2 - keynote lecture

Non-invasive assessment of physiological status: A 30-year contribution to primatology

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The ability to assess physiological status is important in helping to provide a more complete understanding of primate biology. Since primates are generally difficult to handle, potentially dangerous and sensitive to physical and social disruption, science and animal welfare as well as practical considerations emphasise the need for a non-invasive approach. Endocrine methods based on the measurement of hormones and their metabolites in urine and faeces have proven particularly useful since they do not require animal capture and/or restraint; samples can be collected on a frequent basis and for prolonged periods of time and the methods can be potentially applied to any given species. To date endocrine data exist for about 25-30% of the existing primate species and this information stems almost exclusively from the use of non-invasive techniques.
There are three main areas where these methodologies have substantially contributed to primatology: 1) facilitating captive management/ breeding programmes, 2) extending the comparative database and 3) integrating physiology and behaviour in primate field research. The latter has led to a new discipline, field endocrinology, in

which quantitative measures of physiological status are used to gauge the significance of observational data, thereby facilitating the testing of hypotheses concerning the adaptive value of behavioural and morphological traits. This has resulted in new insights into actions of physiological adaptation and behavioural and reproductive processes of wild primates in an evolutionary context. In this talk, the history and application of non-invasive endocrine assessment in primates will be reviewed. Examples from the three areas mentioned above will be presented to illustrate how non-invasive endocrine methodologies have contributed to primatology, with special reference to field-based research.

TR3

Tolerance may lead to loss of tenure in tamarin societies: Use of non-invasive faecal reproductive steroid monitoring and behaviour observations to reveal reproductive and life-history traits in wild female cotton-top tamarins (*Saguinus oedipus*) in Colombia

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Cotton-top tamarins (*Saguinus oedipus*) are a critically endangered primate found only in Colombia. Efforts to conserve this species are centered on developing effective management plans that integrate biological and reproductive information regarding population dynamics and factors that influence their survival through pressures of habitat loss. Here, we report 13 years of data on reproductive and life-history traits for wild female cotton-top tamarins (*Saguinus oedipus*) in Colombia. As part of our long-term field observations on multiple groups, all animals have been individually identified and receive annual exams that include physical and reproductive assessments. Field observations were conducted three to five times weekly by a team of observers to record group composition, inter-group interactions, aggression, births and to collect faecal samples. A total of 8,684 faecal samples were collected from 41 females in 19 family groups over 13 years. Faecal pregnanediol-3-glucuronide and oestrone conjugate (PdG antisera P70; E_1C antisera R522-2, C. Munro, UC Davis, CA) analyses were used to assess reproductive condition of the dominant breeding female, daughters and unrelated or immigrating females through changes in reproductive tenure in each group. Average reproductive tenure was 2.4±2.2 years (range 0.2 to 8.2; n=19). We observed 17 occurrences where the dominant female was evicted from the group. In nine of these cases (52.9%), the dominant female was pregnant when evicted. Daughters remained suppressed until 11.1±0.1 months of age (n=5). However, we observed four instances where mother and daughter were pregnant in the same group. Loss of reproductive tenure was primarily associated with immigration and aggression from unrelated adult females (15 of 17 cases; 88.2%). Of these, eight of the immigrating females were pregnant. Pressures from habitat loss may increase population density and contact with adjacent groups which may impact lifetime reproductive success in wild female cotton-top tamarins.

Authors confirm that research conducted followed international, national and/or institutional guidelines for humane animal treatment, and was in compliance with relevant legislation.

TR4

The evaluation of reproductive hormones to determine the efficacy of commercial GnRF vaccines as effective contraceptive options in captive female Asian elephants

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Nulliparous captive female elephants can become more prone to reproductive tract pathologies, presumably due to decades of continuous ovarian activity, or asymmetric reproductive aging. Contraceptive treatment in elephants is not only useful for preventing unwanted pregnancies, but also for managing or minimizing the risk of reproductive

tract pathologies in aging females. Unfortunately, information regarding the optimal dose rate and frequency of contraception options for long-term use is still lacking. In this study, one mid-aged (DOB 1973) and three aged (> 50 years) Asian elephants, housed in Australian zoological institutions were given a series of either Equity® (n=1) or Improvac® (n=3) (commercial equine and porcine gonadotropin releasing hormone (GnRH) vaccines respectively) injections to down - regulate the cyclic production of reproductive hormones. Either serum (n=1 elephant) or urinary (n=3 elephants) progestagen concentrations were evaluated by enzyme-immunoassay throughout the study (2-4 samples per month), starting a minimum of 5 months prior to the contraception trials. The first set of injections (three 2 ml Equity® injections over one month n=1; or two 2 ml Improvac® injections one month apart n=3) did not elicit a change in progestagen concentrations in three cycling elephants and appeared to induce an oestrous cycle in the fourth presumed acyclic female. Subsequent injections suppressed significant signs of ovarian activity for minimum of 10 months in all four elephants. The longitudinal monitoring (3-5 yr) of serum and urinary progestagen concentrations was useful to confirm ovarian activity and monitor the efficacy of the vaccine injections. Although temporary and therefore requiring regular vaccination to achieve reproductive quiescence, both Equity® and Improvac® appear to be useful methods to suppress oestrous cycles in Asian elephants. The temporary nature of these vaccines suggest this method of contraception may be useful to manage periods between desired pregnancies in elephants, increasing inter-calving intervals without extended periods of cycling reproductive hormones. These results also highlight the importance of monitoring hormones throughout contraception trials.

TR5

The use of altrenogest to reduce ovarian activity and avoid hyperoestrogenism after eCG-hCG ovulation protocol in tigrina (*Leopardus tigrinus*)

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The goal of this study was to develop a method to assist artificial insemination (AI) in the endangered tigrina (*Leopardus tigrinus*), a small tropical spotted felid. The specific aim was to demonstrate the efficacy of altrenogest, an oral progestin (Regumate®, Intervet/Schering-Plough Animal Health), in reducing ovarian activity and hyperestrogenism episodes after administration of exogenous equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG). The most common cause for low pregnancy rate after the administration of gonadotrophins is ovarian hyperstimulation. This may lead to high levels of oestrogen, premature or excessive progesterone release or even to luteal insufficiency, causing premature luteolysis. To achieve the objectives of this study, faecal oestradiol and progesterone metabolites were quantified by an enzyme immunoassay in six tigrina females before and after (i) the i.m. administration of exogenous gonadotrophins eCG (200 IU) and hCG (150 IU) and (ii) the i.m. administration of exogenous gonadotrophins eCG (200 IU) and hCG (150 IU) preceded by the administration of altrenogest, for 14 days (minimum of 0.192 mg/kg/day). Within an individual, with the administration of altrenogest prior to eCG/hCG protocols a significant ($p<0.05$) decrease in oestrogen concentrations was observed in four out of six females after the gonadotropin protocol. Two out of six individuals also showed a significant ($p<0.05$) reduction in progesterone concentrations. Oestrogen concentrations where close to baseline in 50% of these individuals following altrenogest and eCG/hCG treatments. This study demonstrated the efficacy of using altrenogest in tigrina to reduce ovarian activity and avoid hyper-estrogenism after administration of eCG and hCG. However, more studies are urgently needed to test other altrenogest concentrations and verify the efficacy of this protocol in elevating pregnancy rates through AI in this species.

TR6

Seasonal hormonal adaptations in felid reproduction

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The effect of season on sexual hormones is necessary to consider for the breeding and conservation of rare felid species. We compared gonadal hormones in two felid species to determine if varying photoperiod affected their reproductive biology.
The domestic cat (*Felis catus*, Fc) has been described as a non-seasonal breeder when found near the equator. The leopard cat (*Felis bengalensis*) breeds throughout the year in tropical Asia. The far-east cat (*Prionailurus bengalensis euptilura*, Pbe), a subspecies of leopard cat, inhabits forestry landscape in temperate climate. Reproductive activity has not been previously studied in this subspecies. The study was conducted at a research station situated 60 km northeast from Moscow. We monitored reproductive activity of Pbe (n=6 males, 5 females) and Fc (n=5 males, 7 females) from 2006 to 2011. Faecal samples were collected weekly during one year. All the animals were kept outdoors in nature light. The measurement of hormonal concentrations in faecal samples was conducted using commercial EIA kits for testosterone in males and progesterone and oestradiol in females ('Immunotech', 'Chema-Medika', Moscow, Russia). We observed seasonal changes in gonadal hormones in both species [Friedman ANOVA: testosterone (Fc: p=0.02; Pbe: p=0.17); oestradiol (Fc: p=0.0005; Pbe: p=0.02); progesterone (Fc: p=0.0004; Pbe: p=0.006) across seasons]. There were species differences in seasonality observed. Specifically, testosterone peaked in spring in Pbe males, but concentrations remained elevated during spring and summer in tomcats. An oestradiol peak was noted in spring in females of both species, but oestradiol remained high in Pbe females during the summer. Additionally, progesterone was elevated in summer in females of both species, but Pbe females had high progesterone level in spring as well. Thus we showed two important facts: faecal hormonal concentration was influenced by season in both species and there were differences in seasonal hormonal profiles between these species. These findings may indicate variations in mechanisms of hormonal control of reproduction, pregnancy and lactation in these species.

TR7

Faecal hormone metabolite monitoring of reproductive patterns and seasonality in red river hogs (*Potamochoerus porcus*)

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The red river hog is a species of *Suidae* native to equatorial Africa. Very few studies have been conducted on red river hog reproductive biology in captivity. Furthermore, in spite of breeding efforts of various zoos, success has been relatively poor, particularly in the North American captive population. The main study objectives regarding red river hog physiology include: 1) Determining the general patterns of oestrous cyclicity in North American captive red river hogs, 2) Assessing, if present, the factors associated with captive North American red river hog seasonality, and 3) Comparing male and female patterns of reproductive hormone secretion.
In this study, we used faecal hormone metabolite monitoring to analyse samples collected approximately daily from two males and three females housed under natural light over eight years to gain insight into their patterns of reproductive hormone secretion. Faecal hormone metabolites were extracted using 80% ethanol. A progesterone and testosterone enzymeimmunoassay (EIA) were validated and used to monitor reproductive patterns, seasonality, and ovulatory activity including one successful pregnancy. The findings indicate that female red river hogs are a seasonally polyoestrous species. Regular cycles were observed from approximately December through August and an annual period of anoestrus was observed from approximately September until December. Average cycle length for all females was 23±1.19 days. Androgen metabolite excretion patterns of the two males did not show clear seasonal patterns. Only one male experienced an increase in androgen levels corresponding with the female

seasonal oestrous period. However, there was some evidence of possible androgen suppression between the two males, and a potential 'boar effect' on a young female upon first introduction to a male. Ultimately, this information may increase our understanding of this species reproductive biology and serve as a baseline for more in-depth follow-up studies to identify specific patterns associated with reproductive success.

TMe1 – keynote lecture

Remnants of stress hormones: Measurement and biological activity

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The front-line hormones of the body to overcome stressful events are the glucocorticoids and catecholamines. The term glucocorticoid covers a group of substances which bind to the glucocorticoid receptors. Most of these substances are produced by the adrenal glands, but other organs like the lymphoid tissues, the digestive tract or the skin can also form cortisol or corticosterone.
In many species glucocorticoid concentrations in blood show a circadian rhythm, but ultradian patterns also play a role in the effects of these hormones. Monitoring short term variations demands frequent blood sampling, which will increase the stress level in non-habituated animals. Alternatively, glucocorticoids can also be measured in saliva. Before elimination via urine and faeces the glucocorticoids are metabolized into a multitude of metabolites, and in the gut, microorganisms are further converting these steroids. Therefore analytical tools are needed to detect the remnants. These substances can be measured either by immunoassays or physical/chemical methods such as chromatography plus mass spectroscopy. The first challenge is to find the best matrix for a given aim to analyse the stress hormone metabolites and to find a well-suited detection system. The second challenge is to select the timing of sample collection and the storing system, esp. in field endocrinology. Different immunoassays have unequal cross-reactions with glucocorticoid metabolites. The specificity of an assay can be validated using immunograms and results have to be considered as 'equivalents' of the standard used.
Concerning the biological activity of the glucocorticoid metabolites, only limited information is available. A part of the glucocorticoids undergoes side chain cleavage and resulting metabolites act in fish as androgens, as they resemble 11-ketotestosterone, a dominating fish androgen. One of the cortisol metabolites (11-oxoaetiocholanolone) is described as a putative pheromone. Therefore, the concentrations of some glucocorticoid metabolites in effluents of areas of high animal density and of sewage treatment plants may have some importance for aquatic organisms.

TMe2 - keynote lecture

LC-MS analyses of steroid hormones or their metabolites from faeces and urine: Problems and perspectives

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Within the last decades, measuring steroid hormones in non-invasively collected samples such as urine or faeces has become a powerful tool providing important information about an animal's endocrine status. Group specific immunoassays have been developed and checklists created to assure a proper validation of specific assays for different species and matrices. Validations often necessitate invasive experiments such as challenge studies or the administration of radioactively labeled hormones to assure that measurements achieved with the assay correspond to the hormone of interest. Such validations are problematic for use on endangered species where invasive administration of hormones is not allowed. Here a comparison of measured values in immunoassays with the ones in LC-MS/MS can be helpful, to explore if it is indeed the targeted hormones or their metabolites that are responsible for the variation observed in the measures received with immunoassays. Furthermore, LC-MS/MS measurements can be of major interest when a simultaneous quantification of a high number of steroids is needed, for example for the monitoring of cross-talk between the gonadal and the adrenal axis. Here we present an overview of general principles

of LC-MS/MS measurements, considerations for sample extraction and examples of recent applications. We furthermore give estimates of the costs for the purchase, maintenance of LC-MS/MS systems and of sample measurements.

TMe3

Steroid extraction: Get the best out of faecal samples

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Measurements of steroid hormone metabolites in faecal samples as non-invasive parameters for reproductive functions and stress hormone levels have become increasingly popular. The extraction of these steroids from the faecal matrix represents the initial step before quantification by different immunoassay techniques can be performed. The steroid metabolites present in the faecal matrix are of varying polarity and composition. Therefore the selection of a proper extraction procedure is essential. Furthermore, extraction should be kept as simple as possible, because additional steps increase the variation of determined concentrations. Some studies have already dealt with this complex but rather unnoticed matter. Radiolabelled steroids (e.g. cortisol or progesterone) were added to faecal samples to estimate the efficiency of used extraction procedures. However the added steroids are normally not present in the faeces and therefore results are artificial and do not accurately reflect the actual recoveries. In this respect recovery experiments based on faecal samples of radiometabolism studies are more helpful. Their metabolite content reflects the mixture of GC metabolites actually present in the given species very accurately. Consequently, in this study the evaluation of different extraction methods in faecal samples of sheep, horses, pigs, dogs (^{14}C-steroids) and mice (^{3}H-corticosterone) utilized samples containing the naturally metabolized, radiolabelled steroids. Based on our results, we recommend extracting faecal steroids by simply suspending the faeces in a high percentage of alcohol (for glucocorticoid metabolites 80% methanol proved best suited for virtually all mammals tested so far). This significantly increased total radioactivity recovered, but also the relative portion of unconjugated metabolites, which are more likely to be recognized by the antibodies used in various immunoassays. Therefore the advantages of this extraction procedure are plain: it is easy to use (no evaporation step is needed), yields high recoveries and variation based on the extraction procedure is reduced to a minimum.

TMe4

A practical field extraction method for non-invasive monitoring of hormonal activity in the black rhinoceros, *Diceros bicornis*

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Non-invasive hormone analysis is a vital tool in assessing animal welfare and reproductive status, and is commonly utilized in zoos to improve breeding success and animal husbandry. However, it becomes harder to employ these techniques away from controlled laboratory conditions. Field researchers may find themselves without access to reliable electricity, which poses a problem when faecal samples need to be frozen to prevent sample degradation and stored for extended periods. The aim of this study was to develop a field-friendly technique for non-invasive monitoring of hormonal activity in the black rhinoceros, *Diceros bicornis*. Faecal samples were collected from male and female black rhinos, and a field extraction protocol developed which comprises two parts. The first stage allows the extraction of hormone metabolites from a faecal sample under field conditions; this extract is then loaded onto a C-8 Hypersep solid-phase extraction cartridge (Thermo Scientific) which can be easily stored in the field. The second stage of extraction can then continue back in the laboratory following the normal protocol for processing faecal extracts. This technique was validated on testosterone (R156/7, UC Davis), progesterone (CL425, UC Davis) and corticosterone (CJM006,

UC Davis) assays through parallelism and matrix interference assessment. Samples collected over a six-week period from one male (n=20) and one female (n=20) black rhinoceros were also used to demonstrate comparable results between the current laboratory method and the developed field extraction method. Results from all three hormones remained consistent, even after cartridges had been stored in either cool/dry or warm/humid conditions for 1, 2, 3 and 6 months before re-extraction of hormone metabolites for analysis (n=5 male and n=5 female for each condition). This technique for storing faecal extracts could be applied to a wide variety of species, and allows samples to be stored in the field for up to six months, without degradation of hormone metabolites.

TMe5

The confounding effect of GI activity on reliability of faecal glucocorticoids as biomarkers of stress

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Faecal corticosterone metabolites (FCM) have proven an invaluable tool in evaluating laboratory rodents' response to stressors; capable of estimating HPA axis activity when other measures come up inconclusive. However, some basic questions related to validation of this method remain unanswered. Whereas exogenously administered ACTH will produce a subsequent increase in FCM, will a stressful experience – of a certain duration and magnitude – under all circumstances do the same? And conversely, is an increase in FCM always preceded by elevated levels of circulating glucocorticoids? Falling between assay validations and applied experiments, these questions are often neglected.
When applying FCM as a straight-up metric we have found discrepancies with potentially worrisome consequences. When GI motility/loading is changed by surgery-associated temporary ileus or change in diet, mechanisms currently unaccounted for step in, compromising the reliability of FCM as measures of stress. Expressing FCM per weight of excreted dry mass is especially vulnerable to these effects as this approach inadvertently masks the effects. In recent studies we demonstrated that the stress reaction, post-anaesthesia, in a mouse can be readily detected as an elevation in FCM. Combining the anaesthesia with a surgical procedure capable of inducing ileus will however abolish the elevation in FCM. In a less radical setting we further demonstrated that a change in diet could lead to a 30% reduction in excreted FCM which in turn, expressed per weight of dry mass, could be misinterpreted as more than a 50% increase.
In summary, the controversy over how to usefully express measures of FCM and the radical effect a change of diet can have will be discussed, as well as how surgical procedures may influence FCM, and disqualify these measures in relation to post-surgical pain and distress. Far from having all the answers, perhaps we can offer the right questions to ask.

TMe6

Is stress of the female bird reflected in her eggs?

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Quantification of corticosterone in bird eggs has been performed to non-invasively assess stress and to study effects of maternal stress on offspring. This study focused on validating applied analytical methods. Performing these validations, we found that in egg yolk and albumin of domestic chickens, our in-house corticosterone antibody cross-reacts with other steroids, mostly gestagens. Because of the high concentrations of yolk progesterone, pregnenolone and others, even low cross-reactivities seriously confound corticosterone measurements. These findings were consistent across all bird species examined to date (domestic chickens, jungle fowl, starlings, barn owls, Japanese quail, rockhopper penguins, imperial shags). In addition, we evaluated two commercial corticosterone immunoassays and compared them to the in-house assay. Although percentages of cross-reactivities with progesterone differed greatly between the antibodies, high-performance liquid-chromatographic (HPLC) immunograms of yolk

extracts revealed that both assays mostly measured immunoreactive substances with chromatographic characteristics different from authentic corticosterone. Corticosterone concentrations in bird eggs are therefore much lower than assumed, probably because the ovary degrades corticosterone during egg formation**.** In an *in-vitro* experiment, ovarian tissues converted corticosterone into more polar metabolites. This might be a mechanism to protect developing embryos from glucocorticoid overexposure. Maternal stress might translate to the offspring via alternative pathways: Laying hens (n=20) with artificially elevated plasma corticosterone levels produced eggs with smaller yolks (p=0.009) containing fewer reproductive hormones (yolk progesterone: p=0.019; mean ±sem concentrations 3156±74 ng/g yolk versus 3430±78 ng/g in controls; yolk testosterone: p=0.01; 3±0.1 ng/g versus 3.6±0.1 ng/g in controls). Maternal stress might thus affect offspring via differential accumulation of reproductive hormones in the yolk and/or reduced nutrients. For non-invasive stress assessment in laying females, measuring egg weight or yolk reproductive steroid concentrations seem to be promising tools. Taken together, our findings underline the need to prove exclusive measurement of the substance in question whenever an uncharacterised matrix is analysed.

TMe7

Multiple sampling of common terns via blood-sucking bugs: change of prolactin and corticosterone values during incubation

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The production and incubation of a clutch of eggs in birds is linked with costs for the parents: fathers often have to feed their partners to enable them to produce the eggs, which usually compose a considerable amount of the females own body weight. Measuring hormones like prolactin or corticosterone can provide insights into mechanisms that regulate reproduction due to their link with body condition or breeding behaviour. Elevated prolactin values are necessary for constant incubation, while an increased corticosterone concentration is related to activity or stress. Between 2006 and 2009 we sampled both mates of 57 pairs of common terns (*Sterna hirundo*) repeatedly during their respective early, mid and late incubation. To avoid problems of trap-shyness or haematoma during the multiple sampling, we used a minimally invasive method to take samples: blood-sucking bugs (*Dipetalogaster maximus*) in hollow eggs. Via this method we were able to measure baseline prolactin and corticosterone without trapping the birds. We analysed the influence of breeding experience and sex on prolactin and corticosterone values, taking into account the incubation phase. Prolactin values decreased towards the end of incubation in young pairs but stayed elevated in experienced birds. After hatching, fathers showed a decreased prolactin level but elevated corticosterone values, probably due to their role as feeders, while the mothers stayed with the young in the first days. Inexperienced terns exhibited high corticosterone values also directly after clutch initiation. The influence of breeding experience illustrates that young individuals differ from older ones in how they manage the demanding reproductive season, which is probably linked with varying prolactin or corticosterone values.

TMe8

A novel method for non-invasively determining identity, gender, and reproductive status in vertebrates using Faecal Near Infrared Reflectance Spectroscopy (FNIRS): Case study results from okapi and giant panda

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Current technologies for remote monitoring of an animal's physiological profile in the field require collecting faeces and bringing the samples back to the lab for analyses. Hormone extractions, enzyme immunoassays and genetic analysis can be expensive, time consuming, and labor intensive. In partnership with Mississippi State University, Memphis Zoo scientists have developed a novel field-friendly and rapid method for scanning faecal samples in the wild using Near Infrared Reflectance Spectroscopy (NIRS). Once a calibration library has been developed, an NIRS back-pack unit

can be carried into the field and non-invasively scan faecal samples with real time results within minutes. Traditionally, NIRS has been used to collect information on the diet quality of forage and is just beginning to see application for remote physiological monitoring. The objective of our research is to develop faecal calibration libraries to discriminate identity, gender, and reproductive status in the okapi and giant panda and take this technology to the field. In brief, faecal samples from okapi (n=11) and pandas (n=15) were scanned fresh, frozen-thawed, and at various stages of being dried and ground. NIRS spectra were obtained from faecal samples (n=15-200/individual/species), using an ASD FieldSpec3® NIR Spectrophotometer. Chemometric analysis of spectra was done using the GRAMS9.0© software, while data assessment utilized a 2-block partial least squares 1 discriminate function and proximate component analysis. Our results indicate that gender, identity, and pregnancy discrimination were all correctly predicted greater than 90% of the time in both the okapi and giant panda. In conclusion, FNIRS is a rapidly developing new method for demographics analysis and population monitoring of wildlife in the field. We believe this technology will soon revolutionize how we utilize endocrine measures to answer questions related to an animal's reproductive state.

TS1 – keynote lecture

The role of faecal glucocorticoid metabolite monitoring for welfare assessments and conservation management of wildlife

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In recent years non-invasive faecal glucocorticoid metabolite monitoring (FGM) has gained much popularity as a tool for the assessment of animal stress and welfare. While this technique only provides a single measure of an individual's physiological and psychological response to changes in its environment, it has nevertheless proven very useful for assessing stress responses related to animal transport, handling, exhibit changes, and several other management variables in the farm, lab, and zoo and aquarium setting. More recently FGM monitoring is also being applied to an increasing number of free-ranging species to assess the impact of a wide range of variables, such as predation, relocation, reintroduction, and various forms of human disturbance. However, the technique also has its drawbacks, and careful validation and evaluation on a case-by-case basis are necessary, before drawing conclusions based on collected data. Due to the complexity of the stress response itself, and our still considerable limitations in understanding various aspects of chronic versus acute stress, and adaptive versus non-adaptive responses, great care needs to be taken when interpreting obtained results for management decisions. Using FGM monitoring in combination with other measures is recommended in order to evaluate the overall impact of changes in adrenal activity in a given species and study. An overview of some of the 'pros and cons' of FGM monitoring for welfare studies will be provided together with various examples of its successful application. In particular, several case studies that highlight the importance for integrating multiple measures, in combination with FGM, will be presented. If used judiciously, FGM monitoring can provide a valuable tool to help improve the welfare of animals in captive and wild settings by positively affecting management and conservation decisions.

TS2 - keynote lecture

Non-invasive monitoring of stress hormones in rodents and its application in biomedical research

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Rodents represent the vast majority of all vertebrates used for research worldwide. In particular, mice are extensively utilized as animal models in the biomedical sciences. They are easy to handle and breed, and can be easily kept in large numbers. Moreover, established techniques allow genetic manipulations, generating transgenic and knockout mice that are increasingly used in diverse areas of research. However, due to their small body size and the invasiveness of common blood sampling techniques, the monitoring of endocrine functions is seriously constrained. Measuring steroid hormone

metabolites from faecal samples could overcome this problem.
Using faecal samples offers several advantages such as easy collection and feedback-free sampling, thereby avoiding all stress effects associated with blood sampling. Furthermore, as there is no need to capture and handle the animal, repeated sampling of the same individual is possible without affecting its endocrine status. This allows measuring short-term hormonal changes (e.g. before and after drug treatment or social encounters) as well as long-term monitoring of endocrine profiles (e.g. during the course of a disease or therapeutic treatment). Additionally, the diurnal variation of glucocorticoids can be followed repeatedly in individual animals. Thus, this technique facilitates investigating endocrine features in mice that are hardly detectable using conventional methods and can significantly contribute to the 'Reduction' and 'Refinement' recommended in the 'Three R' concept.
In this talk, I will present our studies performed to develop and extensively validate the measurement of glucocorticoid metabolites in faecal samples of mice and also highlight studies applying this non-invasive technique to monitor stress hormones as a powerful tool in biomedical and animal welfare research. These insights about important factors influencing the metabolism and excretion of glucocorticoids will also be of high value for other rodent species and can be crucial for appropriately designing studies in captivity and in the field.

TS3

The effect of a fission-fusion zoo housing system on hormonal indicators of stress in Bornean orangutans (*Pongo pygmaeus*)

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In the wild, orangutans live in a fission-fusion social system and show, compared to other great apes, a much more solitary lifestyle. However, most zoos keep them in unnatural permanent groups which may represent a social and physiological stressor to the animals. In contrast, Apenheul Primate Park in Apeldoorn, the Netherlands, houses 14 Bornean orangutans in four different sub-enclosures in frequently varying groups simulating a fission-fusion social system like in the wild. We studied how this system affected hormonal indicators of stress. Faecal glucocorticoid metabolites (fGCM) were measured with a validated cortisol metabolite enzyme immunoassay to investigate the effect of adult group size, visitor number, sex, age, and change of group composition on fGCM levels. In addition, we compared fGCM levels of the Apenheul animals to those generated in an earlier study from orangutans kept in permanent groups (number of groups 17, range of individuals per group 1-5). We found that fGCM levels increased significantly with visitor numbers, but were unrelated to the sex and age of the animals and not affected by changes of group composition. The Apenheul animals housed in a fission-fusion system showed no increase of fGCM measures when housed in larger groups. By contrast, fGCM levels of Bornean orangutans in 16 other European zoos kept conventionally in permanent groups were significantly higher when groups were larger. Thus, living permanently with several adults seems to increase glucocorticoid output in conventionally kept Bornean orangutans. The approach of Apenheul zoo seems to reduce this group size effect on adrenal activity. However, large number of visitors appear to increase glucocorticoid output of Bornean orangutans housed in Apenheul.

TS4

Hormonal stress response to capture in free-ranging pampas deer (*Ozotoceros bezoarticus*)

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Cervids have recognized agile and violent behaviour as response to stressors and they often suffer severe physiologic alterations during handling. The aim of present study was to evaluate

concentrations of faecal glucocorticoid metabolites (FGM) after capture stress in free-ranging pampas deer (*Ozotoceros bezoarticus*). Twenty adult deer, 11 females and nine males, were captured at South Pantanal wetland (Brazil) in the afternoon and received VHF radio-collars. Untreated adult deer of the same sex were randomly chosen as control group. At the post-capture day an observer followed all animals for faeces collection. Faecal samples were immediately stored in plastic bags after collection and refrigerated up to 12 hours in a thermic bag with recycled ice and then frozen at -20 °C. The faecal samples were lyophilized and homogenized. Following extraction (5 ml of 80% methanol added to 0.5 g dried faeces) FGM were measured using an 11-oxoaetiocholanolone enzyme immunoassay. Data were analysed by repeated measures ANOVA followed by Tukey and P values <0.05 were considered significant. FGM significantly increased ($p<0.01$) from 19-22 h after capture onwards in marked deer and peak concentrations were 2 to 17 times (median: 5) higher as the respective baseline. FGM values of marked deer were significantly higher ($p<0.01$) at 22-25 h and 25-28 h compared with control deer. Marked male deer had significant higher FGM values at 22-25 h and 25-28 h compared with their baseline values ($p<0.01$). However, in marked female deer, FGM values were not significantly different from the baseline. FGM concentrations remained unchanged when stored in thermal bags up to 16 hours. The absences of significant increases of FGM in the captured female pampas deer may indicate that females are less prone to capture stress.

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This study was approved by the Animal Ethics and Welfare Committee (CEBEA) of the Faculty of Agriculture and Veterinary Sciences (FCAV - Faculdade de Ciências Agrárias e Veterinárias), UNESP, Jaboticabal - SP, Brazil (Protocol number: 015077/09).

TS5

Living with the threat of predation: Faecal corticosterone metabolites in bison

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Threat of predation can cause increased adrenal secretion of glucocorticoids, and this elevated stress response might predispose animals to succumb to disease. This study measured faecal corticosterone metabolite concentrations (FCM) using enzyme-linked immunoassay among two bison herds: one that had high numbers of individuals infected with *Mycobacterium bovis* and that cohabitated with wolves, and one that lived without the threat of predation. Samples analysed to date were collected from September through February, which includes the end of the breeding season but does not include the calving season. A group of 20 healthy bison from another geographically remote herd were moved though a chute system, and FCM were significantly higher ($p<0.05$) during this challenge event compared to before and after the challenge, providing physiological validation for the assay. The proportional difference in FCM between samples extracted using a wet-weight protocol and samples extracted using a lyophilization protocol was significantly different ($p=0.02$), indicating that lyophilization was necessary in order to legitimately compare FCM between the two herds. A subset of males (n=10) managed intensively displayed higher FCM ($p<0.001$; 2.1±0.2 µg/g) than similarly-managed females (n=10; 0.6± 0.03 µg/g), possibly indicative of social-sexual differences in that females, but not males, prefer to herd closely together. During September and November, FCM were not different ($p>0.05$) between sick (n=22; 3.1±0.3 µg/g) and healthy (n=30; 2.7±0.2 µg/g) bison cohabitating with wolves but were significantly higher ($p<0.05$) than FCM among bison living without wolves (n=81; 1.6±0.08 µg/g). Interestingly, FCM declined significantly ($p<0.05$) after the fall in both bison herds, possibly indicating the end of the breeding season, and during this time FCM were not different ($p>0.05$) between the herds. These preliminary findings suggest a complex interplay of stressors related to social-sexual differences, season and threat of predation in bison herds.

We confirm that this research followed institutional guidelines for humane animal treatment, and was in compliance with relevant legislation.

TS6

Do stereotypic horses cope better with poor environmental conditions? A physiological approach

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Stereotypic behaviours, *i.e.* repetitive, unvarying and apparently functionless behaviour patterns, are intriguing as they occur in a variety of domestic / captive species without any clear adaptive function. Among different hypotheses, the coping hypothesis predicts that stereotypic behaviours are a way for animals in unfavourable environmental conditions to adapt. As such, they are expected to have a lower physiological stress level (*i.e.* glucocorticoids) than non-stereotypic animals. However, attempts to link stereotypic behaviours with glucocorticoids have yielded contradictory results. Here we investigated correlates of stereotypic behaviours and glucocorticoid levels in two large samples of domestic horses (N_{Study1}=55, 41 geldings, 14 mares, 5-20-year old; N_{Study2}=58 mares, 4-20 year old), all kept for four months (study 2) to at least one year (study 1) in various sub-optimal conditions (*e.g.* confinement, social isolation…) and already known to experience altered welfare states. Each horse was observed in its stall using focal sampling (study 1, 30 minutes in total per horse) and instantaneous scan sampling (study 2, 92 scans per horse). Plasma (collected in study 1; two times per horse between 18:00 and 19:00 over 2 consecutive days) and faecal samples (collected in both studies) were collected to assess cortisol levels. Faecal samples were collected between 12:00 and 13:00 three times per horse on three different days in study 1, once between 08:00 and 10:00 in study 2. Results showed that neither plasma cortisol nor faecal cortisol metabolites concentrations differed between stereotypic and non-stereotypic horses (Mann Whitney tests, p>0.05), nor did they correlate significantly with frequencies of stereotypic behaviours or time spent performing stereotypic behaviours (Spearman correlations tests, p>0.05). As non-invasive measures were performed in both studies, bias due to human interventions during sampling has been minimized. Cortisol measures therefore do not indicate that stereotypic horses cope better, at least in term of basal adrenocortical activity.

TS7

Quantifying fearfulness and aggression in beef cattle using saliva hormone measurements

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Saliva hormone measurements provide, through the combination of non-invasive and integrated collection, values for the free and active form of circulating hormones. Non-invasive techniques are preferred to avoid protocol stress while time accumulated samples resolves the problem of hormone level pulsatility. Fearfulness and aggression in beef cattle are of high importance being linked to poor welfare and productivity. Cortisol is the principle stress hormone in cattle, while testosterone and progesterone have been linked to aggression in male mammals. This study involved taking saliva samples from 120 fattening beef bulls of age range 6 to 24 months. Concentrations of cortisol in conjunction with testosterone and progesterone, were measured in saliva samples using LC-MS/MS detection. The stress response in terms of heart rate and heart rate variability to saliva collection by hand vacuum pump was measured using a Polar heart monitor. It was found that saliva collection was stressful and related to the fearfulness of individual cattle to human approach. Sequential saliva sampling determined that a 15 minute time delay occurred between the acute stress of capture, identified by peak heart rate, and the peak of saliva cortisol concentration. Testosterone levels also increased following the acute stress of capture, while progesterone levels remained static. Both testosterone and progesterone levels were positively correlated to age. These findings are critical to the interpretation of saliva hormone levels for quantifying fearfulness and aggression in beef cattle.

TS8

Can dogs relax? Work-related cortisol levels vary in dogs during animal-assisted interventions: A pilot study

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Positive effects of human-animal contact on human health have contributed to the wide distribution of animal-assisted interventions (AAIs). While considerable effort has been devoted to the research on human welfare associated with AAIs, potential effects on therapeutic animals have received little attention. Therapeutic dogs are required to cope with stressful conditions, deal with unfamiliar people and strange situations. The aim of this study was to determine baseline and work-related levels of cortisol, a glucocorticoid hormone that is known to vary with physiological arousal, in therapeutic dogs. Certified therapy dogs (n=14) aged 5.3±3.9 [AAI programme 1 (AAP1; dogs on-lead during work; n=7) and AAI programme 2 (AAP2; dogs off-lead during work; n=7)] participated in the study. Salivary samples were collected for baseline (at home), pre and post AAI working days and analysed with enzyme immunoassay. Statistics included Friedman two-way ANOVA in evaluating hormone levels and repeated measures ANOVA for working sessions with intervention type as between-group factor and time as repeated factor. There was no difference between baseline cortisol concentrations between dogs working on or off leads (AAP1 5.7±2.4 ng/ml and AAP2 5.0±2.0 ng/ml, respectively). However, significant differences were found between dogs working on or off leads (p=0.010). In the group of dogs that worked off leads (AAP2), the decrease in cortisol from baseline to working levels was -2.45 ng/ml on working day 1 and -2.34 ng/ml on working day 2. In the group of dogs that worked on leads, there was a smaller change in cortisol of -0.59 ng/ml on working day 1 and an increase of +0.28 ng/ml on working day 2. These results suggest that dogs which are off the lead might be less physiologically aroused or more relaxed during animal-assisted interventions.

TNa1 - keynote lecture

Non-invasive pregnancy diagnosis in felids based on faecal prostaglandin F2α metabolites

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In mammals, intrauterine tissues showed a high capacity to synthesize, metabolize and release various prostaglandins (PG) such as PGF2α, which is involved in luteolysis. In canine placenta, PGF2α synthesis is up-regulated after implantation and remained elevated through early and mid-gestation, whereas PGE2-synthases showed a gradual increase and a strong prepartal up-regulation. PGF2α and PGE2 are rapidly metabolized to their metabolites PGFM and PGEM. PGFM has been detected in urine and faeces and can be used for non-invasive pregnancy detection in felids allowing the differentiation from pseudo-pregnancy. The PGFM elevation during last pregnancy trimester has been proved to be valid in six of the eight cat lineages; in some cat species of the Panthera lineage and the domestic cat unclear hormone pattern were described.

By high-performance liquid chromatography (HPLC) immunograms of faecal extracts from different felids, authentic PGFM and several additional prostaglandin metabolites of distinct higher polarity were found to cross-react with our PGFM antibody. Additional LC-MS analyses clearly confirmed the presence of PGFM, but did not result in an identification of any other prostaglandin metabolites.

Several species differences in faecal PGFM metabolite composition were found. In the sand cat, PGFM represents the major faecal metabolite, whereas in lynx it was only a minor component. We also could show that faecal metabolite composition was changing throughout the time course of pregnancy in lynx, probably indicating for changing prostaglandin synthesis and metabolism. Screening faecal samples from lynx with PGF2α - and PGE2

antibodies, however, did not reveal courses of prognostic value.
The physiologic role of PGF2α in felids remains to be elucidated. In the cat, particularly large doses of PGF2α are needed to induce abortion. Therefore, we suggest that the lack of luteolytic capacity of PGF2α is related to other factors that have not been well identified.

TNa2 - keynote lecture

Non-invasive monitoring of hormones in fish

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Fish excrete hormones and their metabolites via the gills, urine and faeces. The methods, advantages, problems and future potential of non-invasive monitoring of hormones in fish via these routes will be discussed. Although steroids can be measured in fish faeces, most workers have measured steroids in the water. Fish release free (non-conjugated) steroids into the water across the gills, which are readily concentrated from fresh- and sea-water samples by solid phase extraction. Water-based measurement can remove sampling effects, enables collection of time series data, and assessment of hormone status of fish that are too small to blood sample. Water can be sampled either directly from holding tanks (non-intrusive and non-invasive) or after confinement in a small volume of clean water for a short (≤30 min) period (non-invasive). Steroid release rates generally correlate with blood steroid concentrations, and the method has proved suitable for studies of stress (by measuring cortisol) and changes in steroids (androgens, oestrogens and progestins) through reproductive cycles. A disadvantage of the water method in comparison to blood sampling is that water hormone concentrations need to be converted to hormone release rates using data on fish biomass, water volume, exposure time and dilution. The direct method is unsuited to situations where the quantities of steroid released are small relative to dilution, or accurate information on fish biomass and dilution is unavailable (e.g. fish farms). Our attempts to find a solution to this obstacle to field application have so far been unsuccessful, i.e. investigation of other metabolites to normalize water hormone concentrations for fish biomass and dilution. Fish do release creatinine and melatonin into the water, but both are affected by feeding rate (and fish feed is a significant source of water creatinine). Nevertheless, non-invasive monitoring of water hormones is proving a powerful procedure in carefully planned experiments.

All experimental studies at Cefas Weymouth were conducted under licenses regulated by the UK's Animals Scientific Procedures Act 1986, and underwent additional peer-review approval by the Local Ethical Review Process.

TNa3

Preliminary study on non-invasive methods for hormonal profile determination in the basking shark (*Cetorhinus maximus*)

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As top predators, sharks occupy a pivotal position in the food chain, yet many species are under increasing threat from fishing pressure and other anthropogenic activities. How these factors impact on their general health is largely unknown, although such information is mandatory for informed conservation management. A hormonal profile could be an important indicator of an individual's general health, yet the principal methods, employing plasma or serum analyses of hormones, are unsuitable for sampling free-living sharks. To produce an easier and less stressful tool to determine the hormonal profile of large sharks we analysed skin mucus for corticosterone and thyroid hormone quantification from the endangered basking shark (*Cetorhinus maximus*, Gunnerus, 1765). Samples were collected from 8 free swimming unstressed sharks as each fed near a stationary boat off the Isle of Man and Southern Ireland, and stored in ethanol. After double extraction in 95% ethanol, samples were dried under a nitrogen stream, and reconstituted with 5% ethanol for assessment of thyroid hormones (T3 and T4) and corticosterone (CORT) using commercial ELISA and Chemiluminescent (CLIA) kits. Mean CORT, T3 and T4 values were 87±81pg/ml, 1.6±2.8ng/ml and 13.8±11.7µg/dl,

respectively. Our results demonstrate that mucus can be used effectively for stress evaluation and is also useful in evaluation of T3, but not T4 levels; the latter was below the limit of quantification in almost 50% of samples. However, as mucus is considered analogous to tissues in terms of hormone concentrations this is perhaps unsurprising, as T4 is generally high in blood and low in tissues. Although preliminary results suggest this method appears to work well with small non-invasive samples readily obtained during field studies, definitive validation will require assessment of hormone levels in a larger number of mucus samples, possibly compared against blood values, from a variety of elasmobranch taxa.

TNa4

Validating thyroxine and triiodothyroxine assays for use in measuring thyroid hormone metabolites excreted in the faeces and urine of the female giant panda.

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One of the greatest challenges in giant panda conservation has been understanding their complex reproductive biology. Although much has been learned about general characteristics of their annual reproductive cycle, little is known about the means by which they achieve reproductive success. Because the giant panda relies on a low-nutrition food source (bamboo), describing metabolic changes during different reproductive states would provide valuable insight into the energetic costs of reproduction. The thyroid gland is an endocrine tissue involved in modulating metabolic rate via thyroxine (T4) and triiodothyronine (T3). These hormones are metabolized and excreted with the faeces and urine, and these substrates have been used to quantify thyroid gland activity in wildlife. We sought to validate thyroid hormone metabolites excreted in the faeces and urine to assess thyroid gland activity, and by extension metabolic activity, in the female giant panda. Utilizing commercially available T3 and T4 assays, we tested displacement of urine and faeces (extracted in 70% ethanol) from a female in late gestation and late lactation. Faecal pools from late pregnancy, and faecal and urine pools during late lactation yielded displacement that paralleled (r^2=0.83-0.89; P=0.01-0.03) standards of the T4 assay, but not on the T3 assay; and urine from late gestation yielded no displacement on either assay. These results demonstrate the first potential to measure thyroid hormones in the excreta of the giant panda. However, the inability to detect T3 and the relatively low displacement (58-77%) of neat urine and faeces on the T4 assay suggest further investigation is needed to develop an approach to accurately and precisely measure thyroid hormone metabolites in the excreta of the female giant panda.

TNa5

Practicalities of urinary c-peptide measurements for monitoring the nutritional status of wild animals

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Nutritional status significantly influences an animal's life, in that its impact ranges from basal metabolic maintenance to behavioural strategies that ensure survival and successful reproduction. Assessing this parameter non-invasively in the wild is important for many behavioural ecologists but has proven difficult. Urinary C-peptide (UCP), a small polypeptide cleaved in an equimolar ratio from proinsulin during insulin production, is a potential candidate in this respect. The use of UCP as a measure of body condition and calorific intake has been validated in several primate species. Nevertheless, the effects of different methods of collection, processing, storage and transport of urine samples on UCP measurements remain unclear. We collected samples from captive macaques, and undertook a series of experiments where we systematically manipulated samples to test the effect on UCP measurements of: 1) contamination of samples on collection; 2) short-term storage on ice; 3) long-term storage methods (blotting onto filter

paper, freezing, lyophilizing); and 4) transportation while keeping samples frozen. Contamination of urine samples by faeces led to a decrease in UCP levels by >90%, but contamination with dirt did not have significant effects. Short-term storage (up to 12 hours) of samples on ice did not affect UCP levels significantly, but medium-term storage (up to 78 hours) did. Freezing and lyophilisation for long-term storage (8 months) did not affect UCP levels, but blotting onto filter paper did. A transportation simulation showed that transporting frozen samples packed in ice and insulated has no significant effect on UCP levels, but only if it can be completed within a period of a few days and if freeze-thaw can be avoided. We use our results to provide practical recommendations for fieldworkers.

TNa6

Peripheral oxytocin levels in wild female chacma baboons fluctuate with oestrous state and involvement in sexual consortships

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The neuro-hypophysial hormone oxytocin (OT) has been implicated in female reproductive and maternal behaviour and in the formation of pair bonds in monogamous species. Here we monitor changes in urinary OT levels across the oestrous cycle and explore the significance of peripheral OT as a non-invasive biomarker of sexual relationships in a promiscuously breeding species, the chacma baboon (*Papio hamadryas ursinus*). Subjects were members of a free-ranging, habituated group of baboons in the Okavango Delta, Botswana. We collected urine samples from n=13 cycling females across their oestrous cycles and during and outside of short-term, exclusive sexual consortships. Samples were analysed via Enzyme Immunoassay (EIA) and non-parametric statistics and a linear mixed model were used to explore the influence of female cycle stage and consortship status, age and rank on peripheral OT. Oxytocin varied significantly across stages of the oestrous cycle, with peak levels occurring during the periovulatory phase. During their periovulatory phase, females in consortships had higher urinary OT levels than females who were not in consortships, although this result did not reach significance. There was also a trend towards an influence of age on peripheral OT levels, which was mainly due to the high OT levels of the oldest female in the sample. Our results indicate that peripheral oxytocin co-fluctuates with changes in reproductive physiology and sexual behaviour in promiscuously breeding wild female baboons. The application of non-invasive methods to monitor peripheral oxytocin presents novel opportunities to examine the endocrine correlates of complex social behaviour across a broad range of species.

TMi1 - keynote lecture

Excreted steroid metabolites in bird droppings: Behavioural studies using baseline patterns and stimulus-specific responses

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The physiological mechanisms underlying behaviour have been studied in birds since the early days of ethology. Measuring hormone metabolites non-invasively is popular with ethologists for a number of reasons. It does not require catching and handling of the birds, which may interfere with ongoing social interactions and measured hormone levels and thus allows repeated sampling. I will emphasize avian studies, which have shown that behaviour may induce substantial hormone changes. In geese, only watching another individual being caught increased circulating corticosterone levels of the spectators. Measuring excreted steroid metabolites from bird droppings is advantageous for monitoring long-term patterns, as well as stimulus-specific responses. We monitored the seasonal patterns of excreted androgen and glucocorticoid metabolites from a free-living flock of the long-term monogamous greylag geese (*Anser anser*) in Grünau. The seasonal baseline patterns differed due to social status, e.g. between singletons, paired and parental geese. Furthermore, the 'within-pair testosterone compatibility' (the degree of co-variation between the male and female pair partners' seasonal androgen patterns) predicted the

pair's reproductive output. In addition to seasonal variation, testosterone may increase in response to sexual or aggressive encounters. Knowing the effective sampling time for dropping collection is essential for studies of stimulus-specific hormonal responses. This may be fiddly with bird droppings because birds excrete urine and faeces jointly via the cloaca. Details on steroid metabolism and excretion kinetics are known for the Japanese quail (*Coturnix japonica*). We have used individual droppings for tests of the post-conflict testosterone changes in staged dyadic fights between male Japanese quails. After fighting, male quail had changed levels of testosterone metabolite and the relationship between testosterone and dominance was considerably affected by social and contextual factors. These data suggest that behavioural interactions, or a single encounter an individual has experienced, can induce hormone changes and vice versa, a single transient hormone surge may affect the individual's future success.

TMi2

Social context: A modulator of behaviour and glucocorticoid secretion

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Presence of conspecifics may lead to increased glucocorticoid secretion (competition, rank), or may have calming effects resulting in reduced glucocorticoid secretion, enhanced exploration or elevated food intake. To examine whether the quality of social relationships and individual behavioural phenotype/personality affect response to social context, we conducted diverse experiments with ravens (*Corvus corax*) and great tits (*Parus major*). In both species, we measured amounts of excreted corticosterone metabolites (CM) in droppings with an 11-oxoaetiocholanolone enzyme immunoassay. In ravens, socio-positive behaviour was related to CM excretion already in nestlings. For subadults, we categorized individuals as fast (FE) and slow (SE) exploratory according to their latency to approach novel objects when alone and observed responses to novel objects when in dyads with siblings and non-siblings. Sibling relationships are dominated by socio-positive interactions. Both exploration style and the social relationship to the companion bird affected exploratory behaviour in social context. To test for potential calming effects of social context after a stressful event we subjected adult female great tits to handling stress. Thereafter we observed behaviour and collected droppings with the mate being either absent or present after females had been handled. On control days females were not handled and remained with their mate. The great tits tested were birds of lines selected for FE and SE behaviour. Females excreted significantly higher CM concentrations when their mate was absent than in the control condition or when he was present (FE: Friedman: df=2, n=6, χ^2=7.0, p=0.03, post-hoc: control<mate absent p<0.05; SE: df=2, n=8, χ^2=4.75, p=0.093). While FE females increased locomotory activity (Friedman: df=2, n=6, χ^2=7.0, p=0.03, post-hoc: mate absent>mate present p<0.05), SE females sat close to their mates longer after handling compared to control days (Wilcoxon: n=8, T^+=32, p=0.054). Our results show that social relationships between the individuals in question and the individuals' coping style/personality are key factors determining how social context affects behaviour and stress hormone secretion.

TMi3

Wildlife management of a highly endangered species: The capercaillie (*Tetrao urogallus*) – implications from behavioural and physiological indicators

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Due to its large spatial requirements and diversely structured habitat needs, Capercaillie (*Tetrao urogallus*) occurrence is on the decline in ever fewer and fragmented patches. The species is

heavily threatened in Central Europe and the study population in the Bohemian Forest (National Parks "Bayerischer Wald" and Šumava and surrounding Landscape Protected Areas) is one of the last in this part of its original range. Presence-absence and abundance data of birds and adrenocortical activity reflected in concentrations of corticosterone metabolites (CM) were measured from droppings. During two consecutive winter seasons 2009-2011 (Nov-Apr) 1,200 droppings were collected. Following field signs (e.g. tracks, foraging places), spatially clustered droppings were assigned to 'individuals' and georeferenced accordingly. Year-round human disturbance, defined as intensity of recreation and forestry activity per grid cell of the study areas, was assessed through expert-survey and tested for influence on habitat use and stress load. Recreation and forestry intensity were not significant factors determining presence-absence, meaning, birds used patches where humans were present. However, intensity of recreation significantly influenced frequency of capercaillie presence in a given plot indicating they prefer patches rating lower in human disturbance. Both recreation and forestry were influential factors in capercaillie stress load (CM values), but were not exclusive in explaining the response variable. Further research into the directness of these factors on resulting endocrine responses is recommended. Habitat quality was an important factor significantly influencing both the behavioural and physiological parameters. It is concluded that a relationship between human disturbances, habitat use and endocrine status does exist. This study underscores the importance of stress ecology research in the context of conservation and wildlife management. Based on the results, capercaillie conservation and management plans should maintain to keep refuges of adequate size and of high habitat quality free from human disturbance, particularly during critical seasons such as winter, lekking and breeding.

TMi4

Difficulties of determining pregnancy non-invasively in the endangered black-footed ferret

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Black-footed ferrets (*Mustela nigripes*; BFF) are induced ovulators requiring pairing or an exogenous hormone injection, such as luteinizing hormone (LH) or human chorionic gonadotropin (hCG), to ovulate. If the female does not become pregnant after ovulation, then she will experience pseudo-pregnancy for the entire gestation (~42 days). Because pregnant and pseudo-pregnant faecal hormone profiles are indistinguishable, our goal is to determine if prostaglandin faecal metabolites (PGFM; 13,14-dihydro-15-keto-PGF 2α) and/or other reproductive hormones can be used to distinguish between pregnancy and pseudo-pregnancy. Oestrus was determined using vaginal cytology analysis in 10 female BFFs. Females were either paired with a male (n=7 females) or given LH (n=2 females) or hCG (n=1 female). Vaginal lavage was used to confirm that all 10 females ovulated. Faecal samples were collected (three/week) from oestrus through gestation. Pregnancy was confirmed by ultrasound at Day 14 and Day 21 in paired females. Two of the paired females were pregnant and subsequently produced kits. However, the hormonal profiles were less conclusive. Pseudo-pregnant females (n=8) had higher ($p<0.05$) overall PGFM and pregnanediol glucuronide (PdG) concentrations than pregnant females. Pregnant BFFs had higher ($p<0.05$) overall testosterone, oestrone conjugate (EC), oestradiol and progesterone metabolites compared to pseudo-pregnant individuals. However, to determine if pregnancy could be predicted before birth, we divided time into three periods: pre-pairing, first 21 days and second 21 days post-ovulation. Unfortunately, all of the hormones were similar ($p>0.05$) between pregnant versus pseudo-pregnant females in the three time categories; therefore, pregnancy could not be determine before the end of gestation. In conclusion, hormonal results have some potential to determine pregnancy, but additional pregnancies are needed to confirm results. While ultrasound was effective and safe, it did require anaesthesia making it more invasive than faecal hormonal analysis. The next step is to try ceruloplasmin analysis to determine pregnancy.

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TMi5

Female signals of fertility and male reproductive behaviour in macaques

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In many primate species living in multimale multifemale groups, females exhibit signals of fertility. There is, however, substantial inter-specific variation in the occurrence of such signals and the selective pressures leading to such variation remain poorly understood. Macaques are an interesting model taxon in this respect since they cover the whole spectrum from absence of obvious signals of fertility to exaggerated ones. Here, we report on results derived from comparative studies on female signals of fertility in free-ranging/wild crested, long-tailed and rhesus macaques. The overall aim of these studies was to determine the function of female sexual signals in these species by identifying the reliability of the signals in terms of conveying information about fertility and by investigating their relationship with male reproductive behaviour. We used patterns of faecal progesterone metabolites to determine the timing of ovulation and female fertile periods. In addition, we carried out behavioural data and signal analysis. We found high inter-specific variation not only in the combination of signals presented, but also in the reliability of indicating fertility of the same signals. Whereas in crested macaques, both female sexual swellings and behaviour indicate fertility quite reliably, only female behaviour does so in long-tailed macaques. In rhesus macaques, sexual swellings are absent, but female facial skin colouration indicates fertility to a certain extent. Male reproductive behaviour also differs between species depending on reliability and combination of signals in the female. We conclude that female signals evolved in order to manipulate male reproductive behaviour and are aligned to species-specific female reproductive strategies. We discuss more in detail the potential causes for the variation found within macaques and in primates in general.

TMi6

Macaque mothers' pre-conception faecal testosterone levels relate to dominance and to sex of offspring

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There is increasing evidence to support hypotheses which suggest that mammalian females may have some influence on which sex of offspring they conceive. In the search for a mechanism by which this might occur, female testosterone has become a focus of interest. Here we report data showing a relationship between level of faecal testosterone in female Barbary macaques (*Macaca sylvanus)*, measured prior to conception, and the subsequent sex of the offspring. Faecal testosterone metabolites we analysed using an enzyme immuneassay. The mean pre-conception maternal testosterone of macaques bearing male offspring (n=4; 154.6±24.4 SEM ng/g) was significantly higher than that of macaques bearing female offspring (n=12; 96.1±16.13 SEM ng/g). Additionally, high testosterone concentrations were also associated with high maternal dominance. Consistent with theories of sex allocation, testosterone has been shown to underpin dominance behaviour and is also likely to be related to good physical and social conditions. In addition, female testosterone, unlike male testosterone, rises in response to chronic stress, thus providing a pathway whereby environmental stressors might influence sex allocation.

TMi7

Endocrine drivers of flexible rutting behaviour in male giraffes *(Giraffa camelopardalis)*

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Males of several species, including the giraffe, adopt a 'roving male' strategy in populations where mates are few and unpredictably distributed. However, very little is known about mating strategies in giraffe, and nothing about potential endocrine control of behavioural tactics used by individual males to deal with the high costs of this strategy. In this study, we used faecal steroid analysis for monitoring androgen levels in free-ranging giraffe bulls to describe the pattern of sexual activity and its endocrine correlates in males of this iconic megaherbivore. From March to May 2011, giraffes were observed for sexual activity for 188 h using 'focal animal sampling' and 'ad libitum sampling' in Hwange National Park, Zimbabwe. 73 bulls were individually identified by their unique pelage pattern and categorized into three developmental stages, Class-A, -B and -C, based on physical appearance, i.e. body size, neck musculature, shape of skull and ossicones. Additionally, 66 faecal samples from 39 different bulls were collected. Dried faeces (~0.1 g) was extracted with 80% ethanol (3 ml) and analysed using an enzyme immunoassay for epiandrosterone (antibody against 5α-androstan-3α-ol-17-one-HS, label: 5α-androstane-3,17-dione-thioether-DADOO-biotin; University of Veterinary Medicine, Vienna). The highest frequency of intersexual activity was observed for Class-A bulls, with the presence of other males stimulating intersexual activity. Class-A bulls have significantly higher median faecal androgen (fA) levels compared to Class-B and -C bulls, and within classes, sexually active males (Class A,B,C: n=8,6,3) had on average higher median fA concentrations than inactive individuals (A,B,C: n=9,10,7). Furthermore, males seem to switch between sexually active/inactive phases at the scale of a week, and their fA levels change accordingly (n=4 cases). These findings suggest that giraffe males have short-term, rut-like periods, but further research is needed to assess the frequency of rutting activity, especially in relation to local male dominance and reproductive success.

TMi8

Carers and non-carers respond differently to a territorial challenge; evidence for a physiological division of labour in the banded mongoose (*Mungos mungo*)

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The specialisation of individual roles within societies is considered a pinnacle of social evolution, yet evidence of proximate mechanisms underlying the division of labour in cooperative vertebrates remains scarce. In non-cooperative species, males typically experience a testosterone surge in response to territorial intrusion that primes them for future aggressive interactions; however, this response is limited to species with no essential paternal care because testosterone typically inhibits parental care in males. These findings suggest that in cooperative species where individuals vary investment in cooperative care there will be a comparative individual variation in testosterone response to territorial challenge; a tentative mechanism to mediate a division of labour between care and aggression within animal societies. Here we use non-invasive hormone sampling and staged territorial intrusions to contrast response to territorial intrusion between carers and non-carers in the cooperatively breeding banded mongoose (*Mungos mungo*). We use linear mixed-models to investigate testosterone response to territorial intrusions whilst controlling for age, sex, weight, and group size and show that territorial intrusion induces a significant testosterone increase in non-carers, but carers show no change in testosterone despite a similar behavioural response to the stimulus (Carers: T1=190.7±26.0 ng/g, T2 =214.0±29.7 ng/g; Non-Carers: T1= 162.5±15.2 ng/g, T2=287.0±35.5 ng/g). Our findings raise the possibility that banded mongooses exhibit a previously unexplored form of division of labour in which non-carers show physiological responses to challenges that leave them better prepared to repel future intruders, while carers do not. While vertebrate societies differ from insect societies in that they lack physical castes, our results provide evidence for more subtle physiologically-based social roles promoting soldier-like and worker-like behaviour in mammals.

TMi9

Adrenarche in bonobos (*Pan paniscus*): Evidence from ontogenetic changes in urinary dehydroepiandrosterone-sulfate (DHEA-S) levels

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Adrenarche is characterized by the onset of adrenal secretions of increasing amounts of dehydroepiandrosterone-sulfate (DHEA-S). While the function of adrenarche remains a matter of speculation, evidence suggests that the morphological and physiological changes related to it are restricted to humans and closely related primates. Within the primate order, adrenarche has only been described in humans and chimpanzees, but bonobos, the sister species of chimpanzees, have not yet been studied regarding early ontogenetic changes such as adrenarche. While bonobos and chimpanzees share many morphological and behavioural characteristics, they differ in a number of behavioural traits and there is a growing interest in terms of the physiological differences that can be linked to species-specific patterns of social behaviour. In this study, we measured urinary DHEA-S levels to determine if bonobos experience physiological changes that are indicative of adrenarche. We validated an ELISA to measure DHEA-S in urine and analysed DHEA-S levels in samples from 53 bonobos ranging from 1-18 years of age. Our results show that bonobos experience an increase in DHEA-S levels after five years of age, which is comparable to the patterns observed in humans and chimpanzees. The differences in DHEA-S levels between the three age categories were significant ($F(2,51) = 30.31$; $p<0.001$). This indicates that bonobos do undergo adrenarche and that the timing of onset is similar for the two *Pan* species. The extraction procedures described in this report demonstrate the utility of urine for monitoring ontogenetic changes in DHEA-S excretion. If applicable to other species, the technique would facilitate more research on the evolutionary origin of adrenarche and other developmental processes.

TMi10

Saliva cortisol in relation to cognition and behaviour after PUFA administration in guinea pigs

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Polyunsaturated fatty acids (PUFAs) are essential nutritional factors involved in maintaining the soundness of neuro-membranes. Some PUFAs (n-3 and n-6 fatty acids) can modulate cognition, social behaviour and the physiological stress response. We investigated the effects of natural foods differing in concentrations of n-3 and n-6 fatty acids in socially living guinea pigs (*Cavia aperea f. porcellus*). Animals (n=40) were divided equally into four treatment groups supplemented with either chia seeds, walnuts, peanuts, or no supplement (control). Influences of nutrients on cognition, social and sexual behaviour were examined using a radial maze task and social confrontation test. Both tests were analysed in relation to saliva cortisol, measured by enzyme immunoassay (EIA) with cross–reactions to relevant 4-pregnene and 5α-pregnane steroids. Saliva was collected by inserting a cotton bud into the animal's cheek pouch for 1 minute. During a 16-day isolation period experimental groups did not differ in daily cortisol concentrations (chia: 30.2±5.0, walnut: 36.2±6.0, peanut: 42.7±7.6, control: 43.5±10.4; mean ± SEM in ng/ml). The maze task revealed positive effects of peanuts (high in n-6) and walnuts (high in n-3 and n-6 fatty acids) on cognition, while no memory effects were detected. However, increasing saliva cortisol concentrations in the retention test were negatively related to latency to bait retrieval and mainly positive to the percentage of movement in PUFA supplemented groups. During the social confrontation test PUFAs did not affect social interactions, while in the beginning fewer sexual interactions were shown by the peanut group. Cortisol concentrations increased significantly in all groups on day 1, which was negatively affected by sexual interactions in the chia (high in n-3 fatty

acids) and peanut groups. In summary the results show the most positive effects on cognition and reduced cortisol concentrations in the peanut group. This seems to be in contrast to former studies, where nutrients high in n-3 fatty acids had positive behavioural and physiological effects, but not n-6 fatty acid rich diets.

PR1

Faecal progestagen patterns in wild African white rhinoceros (*Ceratotherium simum*)

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The white rhinoceros (*Ceratotherium simum*) was rescued from extinction in southern Africa at the end of the 19th century but is still currently listed as 'near threatened' by the IUCN, primarily due to poaching. Captive breeding is a realistic option for long-term survival of the species but, to date, success has been poor. Knowledge of the reproductive status of wild animals can provide valuable information for the development of appropriate management plans. The present study aimed to establish a non-invasive protocol for monitoring faecal progestagen patterns in wild female southern white rhinoceroses. Six adult females at Lapalala Wilderness Reserve, South Africa, were located at least once every week. Three of these six animals gave birth during the study period. Faecal samples were collected at weekly intervals for 12 months (Oct 2008-Nov 2009). Samples were collected fresh and stored frozen at -20°C until analysis. They were lyophilized and approximately 0.05 g of each powdered faecal sample was extracted with 80% ethanol water (3 ml). Faecal extracts were assayed using an enzyme immunoassay for 5*α*-pregnan-3*β*-ol-20-one (polyclonal antibody against 5*α*-pregnan-3*β*-ol-20-one-3-HS-BSA and 5*α*-pregnan-3*β*-ol-20-one-3-HS-peroxidase label). Mean faecal pregnane concentrations in each of the three pregnant females were 35 to 64-fold higher during pregnancy (55-145 days before parturition) than postpartum. The remaining three animals had mean faecal pregnane concentrations comparable to postpartum values. Our results show that non-invasive faecal progestagen measurements can provide information on the reproductive status of wild female white rhinoceroses, and be used for the detection of pregnancy in wild individuals. Collectively, the data clearly underlines the value of non-invasive hormone measurements as a tool to provide information on the reproductive patterns of free-ranging white rhinoceroses, thereby opening new opportunities to optimize breeding efforts of white rhinoceros populations in especially small and medium sized game reserves.

PR2

Reproductive steroid monitoring in white rhinoceroses kept in European zoos

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White rhinoceroses within the EEP are not self-sustaining, as only 20% breed successfully. A comprehensive analysis of this worrying situation was summarized in the 2009 and 2011 in the European White Rhinoceros Studbook. Over the past two decades, faecal steroid hormone analysis in female white rhinoceroses, sometimes accomplished by rectal ultrasonographic examinations, has been used to investigate reproduction and reproductive problems. The 'normal' oestrous cycle is about 35 days in length, however cycles of 70 days in length, as well as missing ovarian activity ('flatliners'), or persistent luteal activity are common observations. Conceptions were observed following oestrous cycles of 35 days. Recently several young rhino cows were imported from South Africa into the EEP. Reaching puberty between 4-6 years of age; oestrous cycles in these young cows usually are 35

days in length. All efforts should be undertaken to successfully breed female white rhinos before age 10 y. Otherwise ovarian activity is prone to the development of persistent luteal activity, paving the way to ovarian and uterine pathologies. In some young cows ovarian activity diminish to flatliner status. The distribution of births in the EEP indicates reproductive seasonality in some cows, which correlates with missing luteal activity during winter months. The EEP recommendation is to keep white rhinos in groups of 1 male and 2-4 females. However this does not guarantee successful breeding. Therefore, in order to stimulate breeding in non-reproducing white rhinos, changing group composition is recommended. Suggestions include transfer of males or females between groups, or to separate, yet to unify males with his herd of females after a few months of separation. Future endocrine monitoring will study effects of varying group size on reproductive parameters, or the influence of a white rhino cow on the reproductive endocrinology of its calf. The ultimate goal is to improve breeding success in captive white rhinoceroses.

PR3

Using hormone analysis to investigate reproductive success in the female eastern black rhinoceros (*Diceros bicornis michaeli*)

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With fewer than 750 eastern black rhinoceros left in the wild, ex situ populations play a vital role in the conservation of this species. However, for captive populations to fulfill their role, they must be self-sustaining. Over the last decade, the European captive population has been relatively stable, but low rates of reproduction are limiting growth. On average, only 11% of mature females reproduce each year and 41% of reproductive age individuals in the current population are yet to successfully produce offspring.
In an attempt to improve population performance, this study was initiated to investigate why individuals may be failing to reproduce. Reproductive monitoring was carried out on 89% of the population (n=24 males, 39 females), using progesterone (CL425, UC Davis), oestradiol (R4972, UC Davis), testosterone (R156/7, UC Davis) and corticosterone (CJM006, UC Davis) enzyme immunoassays to investigate differences in reproductive success.
Reproductive cycles were observed in 94% of mature females, and normal cycles ranged from 20-40 days in length. However, irregular cycles were also common, with a high incidence of long (41-179 days) and short (10-19 days) cycles, and periods of acyclicity also seen. Periods of regular and irregular cyclicity were observed in both parous and nulliparous females; and individuals often exhibited both over a 12-month sampling period.
Average faecal glucocorticoid concentration (fGC) did not differ between parous and nulliparous females, so differences in fGC between regular and irregular periods of cyclicity were investigated within individuals. Faecal glucocorticoid metabolites were higher during irregular cycles and during long cycles, compared to both short and normal length cycles and to anoestrus periods.
A better understanding of reproductive function and factors that could affect reproductive success could improve population performance by increasing both reproductive output and the proportion of females that contribute to future breeding success.

PR4

Relationship between management, adrenal activity and reproduction in a captive group of female Asian elephants (*Elephas maximus*)

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Asian elephants in captivity can be prone to reproductive problems including acyclicity and asymmetric reproductive ageing. To maintain healthy and sustainable herds in captivity, a better understanding of potential influences on reproductive cyclicity are warranted. Routine reproductive monitoring carried out on a herd of five reproductive age females revealed that oestrus cycles were

highly synchronized among individuals. However, a 15-week period of acyclicity occurred in three females, coinciding with a number of management changes and the conception of two herd-mates. The aim of this study was to retrospectively investigate whether management factors or the conception of herd mates was associated with increased adrenal activity and subsequent suppression of ovarian activity. Faecal samples were collected every other day in five female captive Asian elephants for a 16-month period, including four potential oestrus cycles. Samples were analysed for faecal glucocorticoid (FGC; CJM006, UC Davis) and progesterone (CL425, UC Davis) metabolites by enzyme immunoassay. Management factors were recorded daily, including husbandry, training and reproductive notes. Data was analysed using Mixed Models for each individual to investigate whether FGC was related to 1) management [matriarch presence, intensity of training and intensity of foot care] or 2) reproduction [individual reproductive status or the presence of other pregnant individuals]. Routine training and foot care were not associated with any change in adrenal activity; however, intensive foot care was associated with an increase in adrenal activity in one female. Matriarch presence influenced adrenal activity in three adult individuals but not the sub-adults. In the three females that exhibited acyclicity there was no consistent relation between FGC and reproductive state; however, the start of the acyclic period coincided with the dominant female's pregnancy and the conception of a second herd mate. This study provided evidence to support prospective management changes to improve care and welfare in a group of Asian elephants.

PR5

Faecal steroid monitoring as a supportive tool for the reproductive management of okapis kept in European zoos

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From the time when serious breeding efforts of captive okapis in zoological institutions started, problems with reproduction have been recorded. About two decades ago, a method for analysing faecal pregnane metabolite levels was established and used to support the reproductive management of the captive okapi population. In the present study hormone profiles from n=31 okapi females were analysed and correlated to records in the International Okapi Studbook (edition 2011). Additionally, the www.theokapi.org online resource was statistically evaluated. We aimed to find possible causes for reproductive problems. Referring to stud book data, inbreeding to a certain extent was identified in 13 of our study females, however, no direct link between high inbreeding an anomalous hormone profiles were detectable. Acyclic periods of various lengths and persisting up to 1.5 years were identified in 11 animals. Special attention was given to the high mortality of juvenile okapis. Of all individuals listed in the International Studbook, 36.5% died before finishing the 3rd year of life. Mortality rate was highest during the first 6 months of life. The most frequent causes of death were abortions (14%) as well as infectious diseases (14%). In summary, no single cause for the reproductive problems was found. We can conclude that low reproductive rates of captive okapi population are multifactorially conditioned. Optimization of the feeding and housing conditions, as well as genetic management seem to be an essential to improve the reproduction of okapis in captivity.

PR6

Reproductive cyclicity in female okapi (*Okapia johnstoni*): the potential role of mate compatability?

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To effectively manage captive populations, a thorough understanding of reproduction can be beneficial. Endocrinology can help gain an insight into reproductive physiology and has been successfully utilized to track cycles and pregnancy in a diverse range of species. The target for the captive population of okapi is to hold between 215-270 individuals worldwide. Currently, only 174 individuals are held in American and European collections, and population growth in the European collection is lagging behind that of the American,

with more reproductively active females needed to sustain population viability. A better understanding of the link between reproductive cyclicity and mate compatibility could provide a useful management tool. Female okapis exhibit reproductive cycles 15-16 days in length, and are thought to reproduce between 3-16 years in captivity. Reproductive monitoring of a female okapi at Chester Zoo was initiated as part of routine management. Daily faecal samples collected over a five-year period were analysed using a progesterone (CL425, UC Davis) enzyme immunoassay. Aged 2.5 years at the start of monitoring, and accompanied by an unproven male, this female showed erratic progesterone metabolite concentration for 2 months, followed by a period of prolonged acyclicity which lasted 6 months. During this period of acyclicity, a second proven male arrived and 3 months later erratic progesterone patterns recommenced, but again was followed by prolonged acyclicity of 6.5 months. Only on arrival of a third proven male, when she was 4 years old, regular cyclicity began within a month and has continued at approximately 2-week intervals for 2 years. It has been well documented that females are the choosy sex when it comes to selecting potential mates. In captivity, the availability of mates may not necessarily mirror mate choices as presented in the wild. However, could mate compatibility influence reproductive cyclicity to such an extent or are these data simply reflective of the onset of puberty? Further exploration in older individuals may shed more light and prove useful in the captive breeding management of the okapi.

PR7

Pregnancy diagnosis in wild equids, the Przewalski's horse (*Equus caballus przewalskii*) and onager (*Equus hemionus onager*)

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Hormone analysis has been used to successfully diagnose pregnancy in a wide range of species, including domestic and wild equids in captive and field situations. In female *Equus caballus*, unconjugated faecal oestrogens and conjugated urinary oestrogens have been used to distinguish pregnant from non-pregnant equids after day 40 and progesterone metabolites have been used to differentiate pregnancy after the first trimester. Complete pregnancies have been tracked using both oestrogens and progesterone in Grevy's (*Equus grevyi*) and Chapman zebras (*Equus quagga chapmani*); however, there is very little complementary data available for pregnancy diagnosis in other exotic equids.
The aim of the current study was to diagnose and track pregnancy to support the reproductive captive management of the Przewalski's horse and onager. Faecal samples were collected from Przewalski's horses (n=3; age 17-28 years) and onagers (n=7; age 3-18 years) as part of a long-term routine diagnostic monitoring project. Faecal samples were analysed using a progesterone (CL425, UC Davis), a conjugated oestrogen (EC522-2, UC Davis) and an oestradiol (R0008, UC Davis) enzyme immunoassay. Over a 5 year period two full-term pregnancies were tracked in the Przewalski's horses, and three full-term pregnancies and 5 incomplete pregnancies (on-going and/or mid-term abortion) were tracked in the onagers. Progesterone metabolite concentrations enabled clear and substantial differences between pregnant and non-pregnant animals after the first trimester and proved useful to track all ten pregnancies in both species. Oestradiol and oestrogen conjugate concentrations were inconsistent. Clear differences between pregnant and non-pregnant individuals were not apparent in all cases, particularly in the Przewalski's horse. In summary, when using faecal samples, monitoring progesterone rather than oestrogen concentrations gave a clearer indication of pregnancy and can be used to diagnose and track pregnancy in the onager and Przewalski's horse.

PR8

Comparison of two different superovulation protocols on ovarian and adrenal activity in the brown brocket deer (*Mazama gouazoubira*)

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Stress is a limiting factor in assisted reproduction in wild animals maintained in captivity. The aims of the

present study were: to evaluate the influence of two different protocols designed to cause superovulation in brown brocket deer, and to evaluate ovulation rate, presence of functional corpora lutea and adrenal activity after treatments. For this, six hinds were assorted into two groups (n=3) and received two different treatments in a cross–over design: the first received CIDR® for 8 days, followed by 0.25mg i.m. injection of OB on Day 8, 700 IU of eCG on Day 4 and 265 µg of PGF2α on Day 0 (Treatment 1) and the second received CIDR® for 7.5 days followed by 0.25 mg i.m. injection of OB on Day 7.5, 130 mg of FSH divided into eight equal doses and 265 µg of PGF2α on Day 0 (Treatment 2). The induced adrenal activity and treatment efficacy were evaluated by observation of CL and analysis of faecal cortisol and progestin concentration (ng/g faeces) at four different time periods [Pre (two days before treatments), Early Treatment (all days during treatments), Late Treatment (the last four days of treatments) and Post (five days after treatments)]. There was no significant difference for faecal cortisol concentration in four different time periods between treatments; however Pre faecal cortisol concentrations were significantly different from Late Treatment within each Treatment [(T1): Pre = 98.5±9.0 vs. Late Treatment = 170.3±23.0 and (T2): Pre = 91.4±10.9 vs. Late Treatment = 190.5±31.8)]. Between the two superovulation protocols, mean faecal progestin concentration and the mean ovulation rate were higher ($p \leq 0.05$) in T1 (4294±769, 6.0±1.7) than in T2 (1571±240, 2.0±0.3). Thus, regardless of the treatment, the influence of the adrenal activity should be considered in the superovulation protocols and, in this case, the eCG seems to promote a better gonadotrophic stimulation to overcome its effects.

PR9

Analytic profile of oestrogens and progestins in different biological matrixes in the ovine (*Ovis aries*)

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The aim of the present work was to evaluate the hormonal profiles of oestrogens and progestins in blood, faeces, urine and saliva during an induced oestrual cycle in ewes. Samples were collected daily for a 60-day period from eight adult (n=8) cycling ewes. The animals were previously submitted to a twelve days protocol of oestrus induction and synchronization. A vaginal sponge (Progespon®, Syntex, Argentina) was placed on day 0 (D0), and removed on D12 together with intra muscular application of 400IU eCG (Novormon®, INTERVET, Brazil). In order to avoid the effect of exogenous hormones, the first cycle immediately after the synchronization was not considered for hormonal analysis. Progestagen concentrations were quantified by two solid phase analytical techniques through radioimmunoassay (RIA) commercial kits (Siemens, Los Angeles, CA, USA) and enzyme immunoassay (EIA) monoclonal antibody CL425 (UCDavis, USA). Oestrogen concentrations were assessed by commercial RIA double Ac (DSL 39100, Diagnostic System Laboratories, Webster, Texas, USA). Correlation in progesterone concentrations were found to be significant for serum and faeces, serum and saliva and faeces and saliva ($r=0.90$, $p<0.0001$; $r=0.90$, $p<0.0001$; $r=0.92$, $p<0.0001$, respectively) during the oestrous cycles (n=15). Oestrogen concentrations in the serum and faeces were also positively correlated ($r=0.74$, $p<0.0001$). Salivary concentrations of oestrogens were not correlated with faecal or serum concentrations of the same hormone. No correlation was found between urinary concentrations and concentrations found in other matrixes for both progestagens and oestrogens. Concentrations of progestagens obtained using RIA and EIA were correlated on faeces ($r=0.78$, $p<0.0001$) and saliva ($r=0.81$, $p<0.0001$). Results indicate that both immunoassays used in the present experiment can be used to evaluate progestagen concentrations on faecal and salivary matrixes during the oestrous cycle of sheep.

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PR10

Assessment of the reproductive physiology of the potto (*Perodicticus potto*)

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Like many prosimians, the potto (*Perodicticus potto*) has been minimally studied. Through non-invasive faecal hormone monitoring, ultrasonography, and behavioural observations, we gathered basic reproductive information from a captive breeding population. Study animals included a young female during transition from adolescence to sexual maturity, adult females throughout the oestrous cycle, mating, and pregnancy, and an adult male paired for breeding. Faecal samples were collected from females every 2-3 days and from the male 1-2 times weekly, dried, and extracted in 50% methanol/PBS buffer solution. Diluted supernatants were assayed for testosterone (T, R 156/7), oestrone conjugate (EC, R522-2), and progestagen (P4, CL-425) metabolite concentrations, with comparisons conducted using t-tests. While baseline P4 of the juvenile female did not differ (p=0.0507) between adolescence and sexual maturity, the irregular excretion pattern observed throughout adolescence transitioned into discernible luteal phases after 18-months of age. Three adult females exhibited luteal phases of two lengths, with means of 19.8±4.03 (mean+SD, n=5 cycles) and 65±2 (n=3 cycles) days. Peak P4 means ranged from 6.04±0.87 to 8.54 ±1.11µg/g faeces. Follicular phases averaged 10.91 ±3.27 days (n=11 cycles) in length and corresponded to observations of breeding. Mean peak EC concentration ranged from 0.12±0.02 to 0.84±0.25 µg/g faeces. Two pregnancies, with a mean gestation length of 169.5 ±0.5 days, occurred during the study. P4 increased steadily throughout pregnancy and averaged 38.03 µg/g faeces. Transabdominal ultrasound confirmed pregnancy at 108 days post-breeding. Faecal T concentrations excreted by the male averaged 4.57 ±1.5 µg/g and did not differ (p=0.081) throughout the year. Observations of copulations and births support non-invasive faecal hormone monitoring and ultrasonography as effective tools in the reproductive assessment of this species, and these data provide a valuable foundation for future potto research.

PR11

Influence of the ovarian cycle stage on female proceptive behaviours and male mating activity in wild tufted capuchin monkeys

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Female tufted capuchin monkeys lack morphological cues of fertility but produce elaborate behavioural displays of sexual proceptivity. Female proceptive behaviours have been proposed to be functionally equivalent to morphological signals of fertility in Old World primates. However, systematic observations on wild populations are not available and the function of these behaviours as signals to males remains unstudied. Therefore, this study examines whether female proceptive behaviours relate to the timing of ovulation and the fertile phase, and whether males use this information to time their mating activities. To address this, we recorded proceptive behaviours and copulations on two wild groups of Argentine tufted capuchin monkeys (*Cebus apella nigritus*). We observed eight cycling females over two consecutive mating seasons for a total of 15 ovulatory cycles consisting of 326 observation-days. Proceptive behaviours and copulations were then compared to the timing of ovulation as determined from faecal hormone analysis. Results showed that proceptive behaviours were significantly associated with female fertile phases ($p<0.001$). Although females targeted dominant males significantly more often than other males, females still mated promiscuously. In addition, copulations occurred almost exclusively during the periovulatory period. Therefore, proceptive behaviours appear to be reliable signals of ovulation and, accordingly, males timed their mating activity around the female fertile phase. Female capuchins may advertise their fertility as a strategy to influence male mating behaviours, potentially allowing them to gain mating-related benefits from males or to manipulate paternity outcome.

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PR12

Non-invasive monitoring of reproductive endocrine profile and socio-sexual behaviour in captive black-tufted marmoset (*Callithrix penicillata*)

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The objective of this study was to describe the endocrine profile and its potential association with socio-sexual behaviour in captive Black-Tufted-Marmoset (*Callithrix penicillata*). The method of choice for was non-invasive monitoring of faecal metabolites of oestradiol (FME), progesterone (FMP) and testosterone (FMT) by enzyme immunoassay of six pairs of captive *C. penicillata*. The samples were collected six days a week, for six months. The results of four females showed a cyclic pattern for the concentrations of FMP with clear peaks followed by sustained high concentrations indicating that ovulation had occurred. The duration of the cycle was 24.3±4.1 days and the follicular and luteal phases lasted 13.04±4.8 days and 11.2±4.2 days, respectively. One female did not show any cyclic pattern during this period but instead sustained high FMP concentration profiles. She was suspected to be pregnant which was confirmed later on. The concentration of FME and FMT showed similar patterns in the cyclic females with higher values in the luteal phase. The pregnant female was monitored until she gave birth, started cycling over after 6 days and became pregnant again after 47 days from parturition, demonstrating the ability to resume cyclic ovarian function during the lactational period as already described for other Callithrichidae. The values for FMT in the males did not show any difference during the studied period. The occurrence of socio-sexual behaviours was observed in both phases (follicular and luteal) showing no differences between them. When the peri-ovulatory period was considered as a third phase, there was evidently higher prevalence of the socio-sexual behavioural variables in this phase compared with the follicular and luteal phase. In conclusion, the profile of faecal steroid metabolites and corresponding socio-sexual behaviour were evaluated in different physiological states such as ovulatory cycles, pregnancy, parturition and lactation. These results validated the non-invasive tool.

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PR13

Reproductive physiology of the world's rarest canid: patterns in oestradiol, progesterone, glucocorticoid and testosterone metabolites in Ethiopian wolves (*Canis simensis)*

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Ethiopian wolves differ from other cooperatively breeding canids in that they combine intense sociality with solitary foraging. In addition, Ethiopian wolves are the world's rarest canid, with less than 500 adults remaining, and reproductive measures such as semen banking and captive breeding have been recommended for their conservation since 1997. Knowledge about Ethiopian wolf reproductive physiology is therefore valuable not only because of their unusual social system as cooperative breeders and solitary foragers, but also because this knowledge could be used to inform future reproductive initiatives for this species. Between 2007 and 2010, we non-invasively studied the reproductive physiology of 20 female and nine male Ethiopian wolves in Ethiopia's Bale Mountains National Park through the collection and assaying of faecal samples (females, n=701, males, n=169). Using a radio and enzyme immunoassays we measured oestradiol, progesterone and glucocorticoid metabolites in

female samples, and testosterone and glucocorticoid metabolites in male samples to answer questions related to social status, reproductive seasonality, and reproductive suppression. We found that dominant but not subordinate females had an oestradiol metabolite surge indicative of oestrus during the annual mating season. However, although no subordinate females came into oestrus during the mating season, two subordinate females showed signs of coming into oestrus outside the mating season, and pseudopregnancy was common in subordinate females. Glucocorticoid metabolite concentrations did not differ significantly between dominant and subordinate females. We did not find seasonal excretion patterns in testosterone metabolites in males, but did find that dominants had higher testosterone and glucocorticoid metabolite concentrations than subordinates, which may be related to higher rates of aggression and mate guarding in dominant males. These data provide the first description of reproductive physiology of Ethiopian wolves. The information gained could be used in future studies about Ethiopian wolf reproductive physiology, and inform future reproductive management initiatives.

PR14

Pregnancy detection and endocrinological regulation of parturition in Tsushima leopard cat (*Prionailurus bengalensis euptilurus*)

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The Tsushima leopard cat is one of the most endangered mammals in Japan, and its captive breeding programme is currently in progress for *ex situ* conservation. Although non-invasive pregnancy detection is one of the important tools to improve captive breeding management, little is known about reproductive endocrinology in the Tsushima leopard cat. The aims of our study were to validate non-invasive faecal hormone analyses as a reliable pregnancy detection method and to demonstrate the endocrinological regulation of parturition with the monitoring of faecal progestagen, oestrogens, cortisol and 13,14-dihydro-15-keto-PGF2α (PGFM). Faecal samples were collected one to seven times a week from female Tsushima leopard cats kept at Japanese zoos. Faecal hormones were measured from 10 days before the last copulation to 10 days after parturition or the end of pseudo-pregnancy. Faecal hormones were extracted with 80% methanol. Faecal steroid metabolites were measured by enzyme immunoassays (EIAs) with progesterone, oestradiol-17β and cortisol antiserum. Faecal PGFM was measured using the PGFM EIA kit (DetectX®, ArborAssays). Faecal progestagen levels remained at the baseline until copulation; the concentrations increased after the last copulation and remained high during pregnancy and pseudo-pregnancy. The durations of progestagen elevation in pseudo-pregnancy were similar to the gestation period (an average of 65.0 days; 4 pregnancies in 2 females). Faecal cortisol and oestrogens were elevated in last trimester of pregnancy. PGFM levels in pregnant females elevated gradually at about 35 days after the last copulation, and declined rapidly after delivery. In contrast, PGFM levels in pseudo-pregnancy remained at the baseline concentration. The determination of pregnancy in the Tsushima leopard cat only using faecal progestagen profiles is difficult, whereas its combination with a PGFM analysis is valuable for reliable pregnancy detection. Our results indicate that the elevation of cortisol, oestrogens and PGFM in the last trimester may reflect the feto-placental stimulation toward parturition.

PR15

Reproductive hormones in serum samples from free ranging Eurasian lynx (*Lynx lynx*) and their value for reproductive assessment

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Felids typically follow a seasonal polyoestrious reproductive cycle, however Eurasian lynx (*Lynx lynx*) demonstrate strong reproductive seasonality with ~one oestrus per year. To investigate whether this phenomenon is related to captivity, samples from free-ranging lynx were collected during (February-May) and outside (June-January) the breeding season. In Norway, lynx pro-oestrus occurs at the end of February or beginning of March and the breeding season during mid to end of March. The Scandinavian lynx project operates in Norway and Sweden, where free-ranging lynx are immobilized as part of a study on lynx ecology. Serum samples of 82 free-ranging individuals (most sampled once n=38, some sampled twice n=14, some sampled ≥ three times n=4) were analysed for oestrogens (E2), progesterone (P4) and the prostaglandin F2α metabolite (PGFM). Mean P4 levels of 5.09 ng/ml were detected between June-January. In pregnant animals, P4 concentrations were elevated, reaching 66.37-144.92 ng/ml by March-May. Before the breeding season (March) elevated E2 levels of 1.46-1.55ng/ml (n=2, along with low P4 levels) might indicate oestrus activity when compared to levels outside breeding season (April-February; mean 0.56 ng/ml). E2 elevation accompanied by high P4 values was detected during pregnancy (n=3, P4=89-144 ng/ml, E2=1.19-1.45 ng/ml). We defined average PGFM basal level in non-pregnant lynx at 5.09 ng/ml. In nine animals (n=3 April, n=5 May, n=1 June) a typical pattern of late pregnancy with elevated P4 (>25 ng/ml) and PGFM (>10 ng/ml) was shown. On a few occasions (n=6, Feb-Mar), high PGFM was noted. We suggest that PGFM in blood indicates a pre-oestrus luteolysis as a prerequisite for ovulation. Pregnancies were confirmed by visiting natal lairs and verifying the presence of kittens in some animals, however in other cases reproductive outcome did not match individual hormone profiles. One explanation might be unknown resorption/abortion rates, which needs to be examined further.

PR16

Non-invasive monitoring of sexual profile in two pubertal captive polar bears (*Ursus maritimus*)

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Little is known about the endocrinology of polar bears, although there is existing data for sun (*Ursus malayanus*) and Formosan bear (*Ursus thibetanus formosanus*). This research aims to evaluate gonadal hormone profiles in two captive pubertal polar bears by monitoring progesterone, oestradiol and testosterone metabolites in faecal samples. Faeces were collected from one male and one female daily for 4 months and stored frozen until analysis. Hormone extraction was performed on 0.3 g homogenized faeces with a double ethanol extraction and analysed with an in-lab RIA method for progesterone (P4), oestradiol (E2) and testosterone (T). Progesterone was also measured using commercial ELISA kits. RIA and ELISA gave comparable results with $p>0.05$. The female was confirmed to have higher concentrations of faecal progesterone compared to the male. Progesterone showed a seasonal fluctuation in both sexes with peak concentrations in March, lower levels during April to early June and then increasing concentrations from June onwards. Male testosterone concentrations were higher than in the female (31.36 ± 17.26 pg/mg vs. 25.33 ± 52.31 pg/mg respectively), while male oestradiol concentrations were lower than in the female (4.64±2.81 pg/mg vs. 6.05±6.61 pg/mg respectively). Male faecal testosterone concentrations demonstrated seasonal fluctuation with increasing and peak values around April and May. These results suggest that faeces can be used as tool for monitoring reproductive profiles in polar bears.

PR17

Validation of enzyme immunoassays for the measurement of reproductive hormones in polar bear (*Ursus maritimus*) urine

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The North American zoological population of polar bears (*Ursus maritimus*) has been declining over

the past two decades and current reproductive rates are not self-sustaining. Longitudinal faecal steroid analysis has been conducted to characterize the reproductive biology of this Arctic ursid. Faecal androgens were useful indicators of oestrus, and progestagen profiles characteristic of a luteal phase and implantation were described, but collective hormone analyses did not allow discrimination between conceptive females and those undergoing pseudopregnancy and/or embryonic loss. Urinary hormone monitoring could complement faecal metabolite monitoring and broaden our understanding of reproductive states by allowing analysis of metabolites more abundant in urine than faeces. Such information may enhance natural breeding success and facilitate the development of assisted breeding technologies. The objective of this study was to validate enzyme immunoassays for detection of luteinizing hormone (LH), oestrogen conjugates (EC), testosterone/androgens (T) and progestagens (Pg) in polar bear urine (PBU). Percent LH binding of a serial dilution of pooled female PBU ranged from 17.1-66.8% (neat to 1 in 16 dilution, r=0.96) and recovery of exogenous LH from PBU was 113.3±7.1% (r^2=0.98). For EC, percent bindings were 56.1-95.3% (neat to 1 in 16 dilution, r=0.97). The recovery of known concentrations of exogenous oestrone-B-glucuronide from PBU was 83.2±5.5% (r^2=0.99). For T, percent bindings were 28.7-84.3% (neat to 1 in 32 dilution, r=0.99) and recovery of exogenous T from PBU was 97.9±3.1% (r^2=0.99). Percent Pg bindings were 15.2-83.5% (neat to 1 in 32 dilution, r=0.97) and recovery of exogenous progesterone from PBU was 100.2±5.4% (r^2=0.99**).** Urinary concentrations of all hormones were significantly elevated above baseline values after treatment of one female with eCG and pLH. Results indicate that the assays are sufficient for measuring these hormones in PBU and longitudinal urinary analysis of cycling female bears is underway.

PR18

Non-invasive monitoring of reproductive hormones in faecal samples from sloth (*Melursus ursinus*) and Andean (*Tremarctos ornatus*) bears, preliminary findings

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Relatively little is known about reproductive hormones in bears. Specifically, there are no published reports of hormone profiles in sloth bears and one limited report on urinary hormones in Andean, or spectacled bears. Faecal samples were collected from two female Sloth bears (ages 9 and 26 y) and one female Andean bear (age 19 y) three times weekly for 12 to 15 months. Samples were lyophilized and extracted with 80% methanol prior to EIA analysis using antibodies against oestrone-3-glucuronide (R522-2 Coralie Munro (CM), all bears), progesterone (CL425 CM, all bears), and testosterone (R156/7 CM, Andean bear). Serially diluted extracts displayed parallelism with the standard curve and >90% recovery of extracts spiked with standard and diluted at least 1:2 in phosphate buffered saline. In sloth bears, detectable peaks in immunoreactive oestrogens occurred on days of observed oestrus and breeding in late July. Subsequent increases in progesterone metabolites indicating pregnancy or pseudo-pregnancy lasted 163 and 193 days in the older and younger female, respectively. Neither female was confirmed to give birth. In the Andean bear, oestrogens gradually increased above baseline in late April and peaked from late July to mid-August coincident with oestrus behaviours and breeding attempts. Thereafter, oestrogens gradually declined, reaching baseline by early October. No discernible patterns in progesterone or testosterone metabolites were detected, and no birth occurred following breeding. This preliminary data indicates oestrogen and progesterone metabolites in faeces provide a reliable indicator of oestrus and method for tracking pregnancy or pseudopregnancy in sloth bears. While oestrogens appeared to signal follicular development and onset of oestrus in the female Andean bear, more research is needed to find suitable methods for hormone monitoring in this species. Analysis of additional progesterone and oestrogen metabolites is currently underway to determine suitability for tracking reproductive events, and additional animals with confirmed pregnancies are needed in future studies.

PR19

Adrenal glands release oestrogens in response to an ACTH challenge: A possible mechanism for stress induced reproductive dysfunction

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Although stress is generally known to induce reproductive dysfunction, the involved mechanisms are not clearly understood. In this study we 1) validate a non-invasive method to assess oestrogen levels in the female golden hamster, 2) investigate the changes in oestrogen concentrations after an ACTH challenge in intact and ovariectomized females, and 3) confirmed the role of the adrenals as an important source of oestrogens in the castrated female by verifying the expression of aromatase (ARO) mRNA in this tissue. Faecal samples were collected from 11 heterogenic, adult (age, 10 wk), sexually mature female hamsters with 3 hours intervals during a 4-day oestrous cycle. Then six females were ovariectomized (OV) and five submitted to a sham procedure (IN). All the faeces voided by each animal were collected daily during 10 days after the surgical procedure and then once a month through seven months. The females were then euthanized and tissue samples (ovary [IN], left adrenal, brain, adipose, liver, gastric mucosa [IN and OV]) were collected from both intact and castrated females. Real time PCR was used to verify the expression of aromatase mRNA in these tissue comparing intact and ovariectomized females. Right adrenals were dissected and weighed. Faecal oestrogens were quantified by enzyme immunoassay. Numerically higher in OV, right adrenal mass was not significantly different between groups. The profile of faecal oestrogens during the oestrous cycle reflected serum oestradiol curves. As expected, oestrogen levels dropped in OV after the ovariectomy, but rose in both groups after ACTH injection. ARO mRNA expression was only detected in ovaries (IN) and in brain and adrenal of both groups. It was higher in OV adrenals. Our results suggest a possible mechanism for stress-induced reproductive dysfunction by the way of oestrogen release by adrenals in response to stress.

PR20

Evaluation of three immunoassay systems for the assessment of urinary biomarkers of ovarian function in the endangered chinchilla (*Chinchilla lanigera*)

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Intensive hunting for fur placed chinchilla populations at the brink of extinction (IUCN critically endangered - Appendix I of CITES). Although native chinchilla are extremely rare, a hybrid has been bred for fur production, providing a unique model to develop procedures that could be applied to their endangered counterparts. Despite its biological and economic importance, little scientific information is available about this species' basic reproductive physiology, a key aspect for the implementation of captive breeding programmes. Attempts to obtain repeated blood samples from chinchillas were unsuccessful because of their stress-susceptible nature. Therefore, non-invasive techniques provided a unique opportunity, allowing long-term endocrine monitoring while avoiding the stress-evoking stimuli of restraint and venipuncture. The objective of the present study was to test the validity and accuracy of different assays systems for quantification of urinary biomarkers of ovarian activity in the chinchilla. Urinary steroid metabolites were assessed in 24 h urine longitudinal samples before and after the injection of eCG (Novormon, Syntex, 30 I.U., n=6). Hormone assays tested included creatinine (colorimetric assay from creatinine standard set, Sigma #C3613), pregnanediol glucuronide (PdG, C. Munro R13904) and oestrogens (EC, C. Munro R522-2) by EIA. Comparative profiles of progesterone (P) and oestradiol (E) metabolites using RIA were also determined (I^{125} RIA kits, Coat-A-Count, Siemens). Finally, same samples were quantified for 17β-oestradiol and progesterone metabolites using an electrochemiluminescence immunoassay (Roche Diagnostics). After eCG injection, elevation of

urinary PdG and EC metabolites above baseline levels occurred after 7 and 9 days respectively, reaching values of 2720±1110 and 22.5±9.7 ng/mg creatinine. Significantly lower metabolite concentrations were detected using RIA for E and P determinations, yet the profiles obtained were similar to those using EIA for EC and PdG. An improved understanding of these aspects will undoubtedly help researchers to use more effective assay systems for the evaluation of reproductive function in this species.

PR21

Ovarian activity in captive and free-ranging European ground squirrels during the breeding and post-breeding period

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Previous studies have documented a second oestrus cycle in female European ground squirrels after they had weaned their offspring, including spontaneous ovulation and an active luteal phase lasting for up to six weeks. Males did not respond to oestrous females during summer because they already had regressed testes and accordingly no matings occurred. These findings were initially obtained from individuals bred and kept in outdoor enclosures within the natural habitat of the species. To gain further information on ovarian activity during the non-breeding phase and to exclude potential artefacts caused by captivity, we compared seasonal changes in faecal progesterone metabolite concentrations (PM) and body mass in female European ground squirrels at five different field sites in Austria. Females were live-trapped, weighed and reproductive status was determined based on teat development and body mass changes. Progesterone metabolites were analysed from faecal samples using a biotin-streptavidin enzyme immunoassay previously validated by analysing simultaneously collected individual plasma and faecal samples. The results revealed two pronounced PM peaks in all studied females. The first peak occurred during gestation, thereafter, PM levels increased again during the weaning phase and remained elevated for about four weeks. Progesterone excretion rates decreased to baseline levels shortly before the onset of hibernation in late August. Pre-hibernation fattening started when post-breeding PM levels had reached peak values and was positively correlated with daily mass increase rates. Based on these results we assume that the extended luteal phase enhances the fattening process prior to hibernation. This is supported by the fact that the course of pre-hibernation fattening in female European ground squirrels is significantly faster than in males, enabling females to immerge into their hibernacula some weeks prior to males, despite the energetic costs of gestation and lactation.

PR22

Non-invasive methods for the hormonal profile determination in eels

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Artificial reproduction (AR) of eel is a growing topic for aquaculture. Monitoring of hormonal profiles during induction of spawning can help in optimizing AR, leading to a higher egg production. A potential non-invasive tool for hormonal monitoring is the outer mucus that has already been used as a medium for vitellogenin detection, a marker of reproductive status and endocrine disruption. In this study, we evaluate hormonal differences in mucus of female (n=9) and male (n=6) eels which underwent different inductive treatments: females with a constant dosage of hormones, females treated with an increasing dosage (Carp pituitary extract for both treatments, 12 injections), treated males (HCG, 5 injections) and a control group for both sexes. Mucus (0,1g) collected after last treatment was double extracted with ethanol. Samples were then dried under a nitrogen stream, reconstituted with 1 ml of ethanol and analysed with ELISA and CLIA tests for thyroid hormones and P4 and E2 (DRG Diagnostic, Germany) determinations respectively. For the T3 analysis, a dilution of 1:10 was necessary due to high levels that were often out of range. Data obtained showed differences between treatments: E2 was higher in control females (102±7 pg/ml) compared to treated ones (46±11 pg/ml with constant dosage and

69±6 pg/ml with increasing dosage); in males, the variation was similar. With respect to T4, the highest female concentrations were in the group treated with a constant dosage (29.52±1.07 µg/dl) while the one with an increasing dosage was quite similar to controls (26.44±1.88 µg/dl and 27.48±2.07 µg/dl); In males the concentrations were 27,48±2,52 µg/dl for the control group and significantly decreased in the treated group (22.58±0.98 µg/dl). Therefore, analysis of eel's mucus is possible and is a very useful non-invasive method for determination of hormonal profiles to monitor health and reproductive status.

PR23

Comparison of hormonal screening results in placenta tissue and blood of *Orcinus orca*.

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Mammalian placentas secrete hormones as an information exchange between mother and foetus. Placental hormones are usually produced in high quantities, to maintain pregnancy and for fetal development, and may play a role in coordinating fetal and maternal metabolism. Few studies about this topic have been done on humans or some domestic animals.
Progesterone (P4) and oestradiol (E2) are necessary for maternal support of foetus survival and development, while thyroid hormones (T3 and T4) have an important role in fetal brain development in the early pregnancy.
This work reports the screening of an orca placenta collected immediately post-partum for the most important reproductive and thyroid hormones, in comparison with a blood sample obtained from the mother during late pregnancy. Chemiluminescence immunoassay (CLIA) and enzyme-linked immunosorbent assay (ELISA) commercial kits (DRG Diagnostic, Germany), were used for the reproductive and thyroid hormones' detection, respectively. Results showed extremely high placental levels of P4, E2 and T3: oestradiol was 200±59 ng/g in blood and reached 1600±63 ng/g in the placenta. Tri-iodothyronine (T3) showed higher concentrations in placental tissue (16.2±12.6 ng/g) than in blood (1.11±0.12 ng/g). Progesterone levels were much higher in placenta (509±29 ng/g) than in blood (2.6±4.12 ng/g), as already described in other mammalian species, but not for orcas. On the contrary, lower levels of thyroxine (T4) were found in placenta (3.14±0.69 ng/g) than in the blood samples (7.98±0.36 ng/g). This is the first study reporting high P4 concentrations in the killer whale placenta, suggesting that this structure is a significant P4-producing organ – next to the Corpus luteum graviditatis – and crucial to maintain pregnancy in this species.

PS1

Ape behaviour and hormonal response to alternation between zoo enclosures at Lincoln Park Zoo

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Recent advances in exhibit design allow for groups of animals to easily move between exhibits. This practice has not been widely studied, but may have a positive impact on the animals' overall well-being. Our objective was to investigate the effect of switching exhibits on the behaviour and hormonal responses of two dominant male chimpanzees and two dominant male gorillas. Behavioural data were collected with instantaneous scan sampling at 30-second intervals for 10 min/hour using an ethogram of 40 behaviours. Faecal samples were collected 3-7 times/week and analysed using Coralie Munro's testosterone (R156/7) and cortisol (R4866) enzyme immunoassay. Each individual served as its own control. For one male gorilla, who experienced the rotation of neighbouring groups, cortisol (FGM) and testosterone (FAM) metabolites remained unchanged ($p>0.05$). For the other male gorilla, FGMs ($p=0.031$) and FAMs ($p=0.002$) decreased when moved and then subsequently returned to his original exhibit. Inactivity was lowest ($p=0.002$) and affiliative behaviour was highest ($p=0.005$) when returning to original exhibit compared to the alternate exhibit. In one male chimpanzee,

who was moved and then subsequently returned to his original exhibit, FGMs and FAMs remained unchanged (p>0.05); however, locomotion was lowest (p=0.017) when returning to the original exhibit and attention to outside stimuli was highest (p=0.031) in the alternate exhibit. In the remaining male chimpanzee, FGMs increased (p=0.027) when moving from the original exhibit to the alternate exhibit and FAMs decreased (p=0.006) when returning to the original exhibit. This individual was also less (p=0.010) inactive and had higher (p=0.06) affiliative behaviour when moving from the original to alternate exhibit. In conclusion, this study highlights: 1) the value of monitoring individuals as there were clear differences between individuals; and 2) switching enclosures may be beneficial in increasing locomotion and affiliative behaviour and has the potential to be used as environmental enrichment.

PS2

Stress levels, dominance rank and hierarchy dynamics in wild male crested macaques (*Macaca nigra*)

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In many animal taxa there is evidence that high social status conveys reproductive benefits. In contrast, costs associated with high social status are much less studied and understood. In male primates, potential costs of high dominance rank are elevated stress levels. This relationship, however, is mostly expected if the social hierarchy is unstable. In this study, we therefore investigate the link between hormonally assessed stress levels and dynamics in rank relationships. On the individual level, we will determine whether changes in glucocorticoid levels differ between individuals that rise as opposed to decline in rank. On the group level, we will test whether during periods of instable hierarchies there is a positive relationship between glucocorticoid levels and dominance rank and whether in stable periods this relationship is reversed. The study was conducted in the Tangkoko Nature Reserve on Sulawesi, Indonesia, on males in two wild groups of crested macaques (*Macaca nigra*). Crested macaques are an ideal study system to address our study questions since rank changes are frequent as are migrations of males between groups and subsequent instable periods. We collected 662 faecal samples from 29 males over the course of 16 months (median: 26 samples per male, range: 1-43). Glucocorticoid levels were assessed by means of a recently validated, 11ß-hydroxyaetiocholanolone assay. Individual rank trajectories and hierarchy stability were derived from Elo-ratings, allowing for a novel way of quantitatively assessing hierarchy dynamics. We will discuss our results in the light of the increasing interest in the relationship between social status and stress physiology. Our results will thereby contribute to the growing body of data on benefits and costs of status and stress in social animals in general and primates in particular.

PS3

Number of fertile females and male social status influence physiological stress levels in wild male crested macaques (*Macaca nigra*)

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In seasonally breeding primates living in multi-male and multi-female groups, males often show an elevation in glucocorticoid concentrations during the mating season due to increased male reproductive competition. The degree to which male competition affects male stress hormone levels in non-seasonally breeding primates remains mainly unknown. In the present study, we examined

the relationship between the degree of male-male competition and glucocorticoid output in wild crested macaques (*Macaca nigra*), a non-seasonally breeding primate. The study was carried out on 22 males from two groups of crested macaques living in the Tangkoko-Batuangus Nature Reserve, North Sulawesi, Indonesia. A total of 2,880 h behavioural observations and 696 faecal samples were collected during the 15-month study. Monthly glucocorticoid concentrations were measured with a validated 11ß-hydroxyaetiocholanolone assay and related to the monthly number of fertile females in the group, individual male copulation, aggressive interaction rate and male dominance rank. Our results show that glucocorticoid levels were significantly influenced by the number of available fertile females (p=0.004) and male dominance rank (p=0.020). Males had significantly lower glucocorticoid levels when many fertile females were available (p=0.004). In contrast, when only few fertile females were available, male-male aggression as well as glucocorticoid levels were increased. Also, high-ranking males had significantly higher stress hormone levels than middle rangking males (p=0.01) and low-ranking males (p=0.016). Our data indicate that in non-seasonally breeding primates, male reproductive competition depends on the distribution of fertile females in the group and that high-ranking males carry the highest costs in terms of physiological stress.

PS4

Relocation stress induced long-term faecal hormonal modifications in semi free-ranging social groups of *Macaca tonkeana*

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A growing body of literature demonstrates the validity of faecal hormonal assessment to evaluate the response to a stress exposure in different mammal species, avoiding the confounding effects of animal manipulations and providing a more accurate estimation of long-term hormonal levels. This approach is particularly useful in free-ranging animals and in group-living animals in which repeated capture and restraint are not feasible. Studies conducted in wild conditions are quite long and difficult to perform but on the other hand, studies in standard laboratory conditions present some limitations. A good compromise consists of studying semi free-ranging social groups of animals living in areas that mimic natural environments. We assessed the effects induced by relocation between two different institutions in semi free-ranging non-human primates (*Macaca tonkeana*). Fresh faecal samples were collected before, during and after the relocation of the animals. Faecal samples were cleaned of environmental substrates, roughly mixed and an aliquot (0.3 g) was weighted and dissolved in ethanol (96%). The solution was homogenized, mixed, boiled at 85°C and then centrifuged (500 x g) at 10°C. The supernatant was collected and the pellet resuspended in ethanol (96%) for a second cold extraction (45°C). The supernatant obtained was added to the previous, dried in a mixer evaporator and resuspended in methanol 100%. Faecal levels of cortisol, testosterone and progesterone were assessed in both male and female animals utilizing EIA commercial kits, modified to avoid the interference of methanol. We observed a clear increase of cortisol and testosterone levels after the relocation that lasted for a variable duration in the weeks following the relocation. After a long period both hormonal levels came back to basal values. We didn't find a clear effect of relocation on progesterone. The results obtained raise further questions regarding the adaptation to social and/or environmental modifications that warrant investigation.

PS5

Feeding competition and stress in wild tufted capuchin monkeys: Implications for the evolution of "deceptive" alarm calling

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An ability to employ tactical deception against conspecifics is argued to have been a factor favouring the evolution of increased encephalization and cognition in primates. However, few systematic observations of deception in wild primates exist, and no previous study has tested whether such behaviours are underpinned by cognitive mechanisms, or can be more parsimoniously explained by non-cognitive mechanisms. Previous research has shown that wild tufted capuchins (*Cebus apella nigritus*) use functionally deceptive false alarm calls to usurp food resources, with false alarms being produced more often: 1) during competitive feeding contexts than in other contexts, 2) when food is clumped (and therefore monopolizable) than when dispersed, and 3) by subordinates than by dominants. This study tests an alternative to the cognitive hypothesis, namely that "deceptive" alarms are underpinned by the production of glucocorticoids, hormones associated with stress. If the stress hypothesis is correct, it was predicted that: 1) competitive feeding would lead to increased stress relative to baseline levels, 2) stress levels would be higher when food was more clumped, and 3) these stress effects would be stronger in subordinates than dominants. This was tested experimentally in Iguazú, Argentina by manipulating within-patch food distribution using feeding platforms filled with banana pieces during periods of low fruit availability. Faecal samples associated with nine alternating 10-day periods of clumped (mean 6.4 samples/individual), baseline (12.8/individual), and dispersed (6.9/individual) conditions were collected from 30 individuals (two social groups) and analyzed for concentrations of glucocorticoids using a corticosterone enzyme immunoassay. Results do not support the stress hypothesis, as stress hormone levels were significantly lower in association with competitive feeding and did not vary based on food distribution or rank. Additional research is needed to determine if "deceptive" alarms are indeed underpinned by cognitive mechanisms.

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PS6

Comparison of faecal cortisol metabolite concentrations in white rhinoceroses (*Ceratotherium simum*) living in three different environments

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Captive conditions may influence animal endocrine physiology, female reproductive cycles, and behaviour. Many efforts are undertaken to minimize animal handling and to improve welfare of animals kept in captivity. The aim of this study was to investigate how habitat conditions, from total captivity to natural habitat, may affect cortisol metabolite concentrations in white rhinoceroses. We studied nine female and six male rhinoceroses. Three rhinoceroses from the Zoo of Madrid were considered to be kept in total captivity; four individuals from the Bioparc of Valencia were considered to live in semi-captivity, because of larger enclosures allowing intra and inter-specific relationships with other savannah species (such as zebras, antelopes and birds); furthermore eight wild rhinoceroses from a South African reserve (Mabula) were considered to live in their natural habitat. Faecal samples were collected early in the morning, twice a week, for approximately one year and were frozen (-20ºC) until analysis. The enzyme immunoassay (EIA) performed used an antibody against 5α-pregnane-3ß,11ß,21-triol-20-one. The technique was validated by HPLC analysis. Since parturition is a natural event resembling a stressful situation, we considered peak faecal cortisol metabolite concentration shown on the day of parturition in one of our females (4,500 ng/g) as a reference value for high concentration. Hormone results indicated

statistically significant differences ($p<0.001$) between samples collected from the three habitats studied, supporting our initial hypothesis. Mean cortisol metabolite values were about three times higher in animals kept in total captivity (1,429±67 ng/g), and also higher in semi captivity (687±13 ng/g), as compared to results from animals in the wild (444±29 ng/g). We also found statistically significant differences ($p<0.001$) between females (1,022±37 ng/g) and males (523±17 ng/g) indicating that females had approximately two times higher cortisol metabolite mean concentrations. We conclude that rhinoceroses kept in total captivity might have higher cortisol levels than animals living under natural conditions.

PS7

Faecal cortisol and progesterone metabolites concentration in four captive white rhinoceros (*Ceratotherium simum*) kept in different housing conditions

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Captive breeding is an important safeguard for the white rhinoceroses (*Ceratotherium simum*), as free ranging populations are increasingly threatened by poaching. However, white rhinoceroses are not breeding well in captivity and many efforts focus on improving captive breeding to increase knowledge about reproductive requirements. Cortisol is frequently used as an indicator of stress, and progesterone is a reliable indicator for monitoring reproductive cycles. The aim of this study was to investigate faecal hormone concentrations of captive white rhinoceroses and to correlate results to housing conditions. We studied four rhinoceroses; three individuals (two females and one male) shared an enclosure, whereas one male displaying a strong stereotypy was housed alone. Behavioural observations suggested a dominant-subordinate relationship between the two females. Faecal samples were collected at 10:00 every day over a year and frozen (-20ºC) until analysis. Faecal cortisol and progesterone metabolites were measured by a 5α-pregnane-3ß,11ß,21-triol-20-one and a 5ß-pregnane-3α-ol-20-one enzyme immunoassay, respectively. The technique was validated by HPLC analysis. Statistical analysis identified significant differences ($p<0.001$) in mean cortisol metabolite concentrations between the two males, being higher in the male living with the two females. Although there were no differences on mean cortisol metabolite concentration between both females, statistically significant differences ($p<0.001$) were found on mean progesterone metabolite concentrations between both females, because the dominant female had oestrus cycles of 29.9±2.13 days in length, whereas the subordinate female was not normally cycling. Results in the two females studied suggested that social status is possibly related to ovarian activity. We found that intra-specific relations between males and females elevated faecal cortisol metabolite concentrations, while hormone concentrations in the single housed male displaying stereotypy were lower. Results of our preliminary study in four animals need to be confirmed in a larger number of animals and under varying social housing conditions.

PS8

Human activity is not the main source of stress in wolves

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Human disturbance has been proven to be a source of stress for several mammalian species and prolonged stress may adversely affect their fitness. The aim of our study was to test if human activity causes chronic stress in wolves (*Canis lupus*). Glucocorticoids in scats have been used as physiological indicators of stress in a variety of species. We therefore assessed the stress level of wolves from six packs (from south-eastern and central Poland) by measuring the concentration of glucocorticoid metabolites in 59 fresh faecal

samples (5-20 samples/pack), collected in the years 2004 - 2007, with a cortisol enzyme immunoassay. Next to human activity (determined by a traffic index combining road density and traffic intensity), we selected pack size (social stress) and prey density (food abundance) as variables potentially affecting stress hormone concentrations. The mean cortisol metabolite concentration of all scats was 11.4±2.8 (95% CI) ng/g wet faeces. During the breeding season (n=40 samples), cortisol metabolite concentration was higher than during the rest of the year (13.4±3.8 ng/g versus 7.1±2.2 ng/g). A multiple regression analysis, corrected for seasonal variation, showed that pack size had the highest impact on the wolves' stress levels (sum of Akaike weights: 0.617), followed by human activity (0.402) and prey density (0.379) within the wolves' home ranges. The results show that reproductive status and social interactions (stress levels increased with pack size) induced more stress than humans did. We therefore conclude that human activity within wolf habitats is only a minor cause of stress in wolves.

PS9

Adrenal activity in captive and free-ranging African wild dogs (*Lycaon pictus*) during the breeding season

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Measuring concentrations of faecal glucocorticoid metabolites (FGM) is a popular method in studies of stress physiology and animal well-being, and has particular value for endangered species that are logistically challenging to study. African wild dogs are highly social endangered carnivores with an obligate breeding system where intensive management has been applied to both captive and free-ranging populations. Information on adrenal response to potential stressors is limited for this species, yet understanding general patterns of FGMs is essential, particularly when trying to identify causes of stress and how this may influence reproductive output. The aims of our study were to: 1) determine whether a commercially available Gamma-coat cortisol RIA can detect a response following a potential stressor; 2) determine which biological factors are associated with high cortisol concentrations; and 3) assess patterns of adrenal activity during the breeding season. Non-invasive faecal samples were collected from 15 captive individuals 1-4 times per week for 2-6 months, and from 62 free-ranging individuals opportunistically collected over two 6 month periods in Hluhluwe-Umfolozi Game Reserve, in addition to behavioural and environmental data. In general, there was a 2 - 3 fold increase in cortisol concentrations above the baseline 24 h after a stressful event such as injury or predator interaction. There were significant differences in cortisol concentrations among zoos in captive populations but not in age, group-housing or reproductive classes. In free-ranging individuals, females had overall higher concentrations than males. Adrenal activity steadily increased with the onset of mating and gestation period in both sexes. We conclude that adrenal activity can be reliably detected using this cortisol assay. The data highlight the value of using non-invasive techniques to assess patterns of adrenal activity during breeding, and should be considered an integrative aspect of management for both *in-situ* and *ex-situ* African wild dog populations.

PS10

Dog appeasing pheromone®: A useful tool to minimize stress and aggression in African wild dogs (*Lycaon pictus*)?

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To preserve a healthy and genetically viable population of free-ranging and captive African wild dogs (AWDs), management actions like translocation and formation of new packs of unrelated individuals are often required. One of the main problems

associated with translocation and subsequently pack formation of AWDs is stress-related intra-pack aggression. Application of Dog-appeasing-pheromone (DAP®) is a widely used approach to reduce stress and anxiety in domestic dogs and could be an equally potential method to reduce stress and aggression in captivity. Therefore the aim of this preliminary study was to determine the effect of DAP® on adrenocortical endocrine function of captive AWD by monitoring faecal glucocorticoid metabolite (FGM) levels. To evaluate whether pharmacologically induced changes in circulating glucocorticoid concentrations are reflected in measured FGM levels, an adrenocorticotropic hormone (ACTH) challenge test was performed on three females (30-35 kg) which were individually housed at the Ann van Dyk Cheetah Centre, RSA. FGM immuno-reactivity was tested using an enzyme immunoassay with an antibody against cortisol-3-CMO raised in rabbit. Administration of 25 IU of synthetic ACTH (Synacthen®, Novartis RSA (Pty) Ltd) per animal resulted in a 2-3-fold elevation in FGM levels after 8-9 hours in all animals. Subsequently FGMs returned to baseline within 3-26 hours. Five days after the physiological challenge, DAP® collars were applied for six days on these three females. Compared to corresponding individual pre-treatment baseline levels, FGM concentrations decreased to 68% and 82% in two of the treated animals. No substantial difference was found for the remaining female. In conclusion, the cortisol-3-CMO EIA provides a reliable method for assessing adrenocortical endocrine function in AWD based on FGM analysis. The promising but inconsistent results regarding the effect of DAP® collars on FGM concentrations, requires further investigation involving larger sample size and animals of both sexes to reveal sufficient evidence for a stress-reducing effect of DAP® in AWDs.

PS11

The use of non-invasive monitoring methods in conservation breeding of the endangered European mink (*Mustela lutreola*)

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Non-invasive monitoring of faecal hormone metabolites has become an established practice supporting many disciplines like veterinary medicine, or conservation biology. Captive breeding of endangered species such as the European mink (*Mustela lutreola*) is confronted with several problems, for instance identifying the right timing to introduce the male to the female. In captive European mink, more than 50% of breeding attempts fail due to aggressive or passive behaviour of the male. In our project, we are investigating several hypotheses to identify reasons for breeding failure: non-physiological hormone cycle, stress caused by husbandry factors, and stress during ontogeny. Non-invasive monitoring of steroid hormones will be of great advantage for the: (1) analysis of faecal progesterone and oestrogen metabolites for determination of oestrus and pregnancy, (2) analysis of faecal testosterone metabolite concentrations for identification of possible physiological factors in non-breeding males, (3) analysis of faecal cortisol metabolite levels for the assessment of stressful situations. We validated and applied the measurement of reproductive and stress hormone metabolites in the European mink. Progesterone and oestrogen levels were found to be significantly higher in pregnant as compared to non-pregnant females ($p<0.001$ for both hormones). Between breeding and non-breeding males no differences were found in faecal testosterone metabolite levels or testicular volumes, suggesting that the development of abnormal breeding behaviour might rather be related to factors caused by stressful husbandry conditions. We are currently investigating stress hormone levels potentially correlated to different husbandry systems and to breeding behaviour; preliminary results will be presented at the conference.

PS12

Individual stress response predicts post-release survival in reintroduced Canada lynx

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Reintroductions are valuable conservation tools, but we have a poor understanding of what factors contribute to their success or failure. We propose that reintroduction outcome may be influenced by individual characteristics of the released animals. In particular, stress coping styles may be a critical determinant of post-release survival. Here, we examine the correlation between individual patterns of adrenal activity and post-release survival in reintroduced Canada lynx (*Lynx canadensis*). Wild lynx were trapped from healthy populations and transported to Colorado, USA, where they were maintained temporarily (1-4 months) in holding pens prior to being released. We monitored patterns of adrenal activity while lynx were in holding pens using a corticosterone assay to quantify faecal glucocorticoid metabolites (FGMs). Intensive monitoring allowed us to develop detailed FGM profiles for each individual. Lynx with a greater increase in FGM following stressors had significantly higher rates of mortality after they were released. However, several other aspects of stress physiology (e.g., mean and baseline FGM) were not correlated with post-release survival. This study enhances our understanding of how reintroduction events impact an individual's stress physiology, and how stress physiology may in turn impact the outcome of the reintroduction.

PS13

Influence of tourism activities on glucocorticoid secretion in mountain hares (*Lepus timidus*)

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Winter human outdoor recreational activities are increasing and have a significant impact on wildlife. There are few methods suitable for investigating the response of rare and endangered species to human recreational activities, although the impact can be assessed at various scales by measuring both physiological and behavioural responses to disturbance. To gain important information about an animal's endocrine status, non-invasive methods for measuring steroid hormone metabolites in the faeces have become a widely accepted tool. Recently such a method was developed for the mountain hare and so we investigated levels of faecal glucocorticoid metabolites (GCM) in the mountain hare in areas that had either no, low, or high tourism activities during winter 2011. Our results showed that GCM secretion was positively correlated with increased tourist activities. We recommend that managers keep forests inhabited by mountain hares free from tourism infrastructure and retain undisturbed forest patches within skiing areas in order to protect mountain hare populations.

PS14

Evaluation of faecal glucocorticoid metabolites in captive red pandas (*Ailurus fulgens*)

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The red panda (*Ailurus fulgens*), a taxonomically unique carnivore and the sole species of the family *Ailuridae*, is listed as 'vulnerable' to extinction on the IUCN Red List. Although the *ex situ* population is essential to securing the species' survival, efforts to improve breeding are hindered by a lack of knowledge about basic red panda biology. Stress plays an important role in the success of animals. Species that are naturally solitary and reclusive like the red panda may be particularly vulnerable to stress in a zoo environment. To enhance our knowledge about the impact of zoo management on red panda welfare, we quantified immunoreactive faecal glucocorticoid metabolites (fGM) concentrations from 26 red pandas at 10 different North American zoos. Eleven (5.6) pandas belonged to the subspecies *Ailurus fulgens styani* and 15 (8.7) to the subspecies *Ailurus fulgens fulgens*. No differences were found in mean fGM concentrations among red

pandas based upon the zoo management factors we evaluated. Animals housed singly excreted similar concentrations of fGC compared to those managed in same- and mixed-sex exhibits. No differences in fGM excretion were observed between bred and unbred individuals. However, fGM concentrations were significantly increased in *A. fulgens styani* compared to *A. fulgens fulgens*. Additionally, all red pandas excreted lower fGM concentrations in the spring compared to all other seasons. Despite the observation of consistent seasonal patterns in all of the individuals assessed in this study, *A. fulgens styani* excreted higher concentrations of fGM than *A. fulgens fulgens* during the breeding (Dec, Feb-Mar) and birth/cub rearing (Jun-Jul) intervals. More work is required to elucidate the significance of subspecies differences in fGM excretion and whether differential adrenal responsiveness to stressors might suggest the implementation of species-specific management strategies to optimize reproduction and well-being in the red panda.

PS15

Response of female veiled chameleons (*Chameleo calyptratus)* in a research colony to environmental disturbances

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Female reptiles are prone to reproductive disorders in captivity. In a research colony of female veiled chameleons (C*hameleo calyptratus*), concern arose when only 1/28 animals laid eggs within the expected timeline; the remainder exhibiting distended, lumpy abdomens for an extended period of time. Bi-weekly ultrasound examinations of the reproductive tract were initiated to assess structures present in the tract (follicles vs. eggs), and determine the time frame for egg laying (based on previous data). Analysis of ultrasound images indicated the presence of large round structures >8 mm in diameter (pre-ovulatory size) on the ovary which remained static, and did not show significant change in size over a 10 week period (well beyond the length of a normal cycle). During establishment of the colony, unforeseen circumstances required the animals be moved to a new environment, including a new room layout, and (other) animals in adjacent pens. The move occurred when the majority of the animals had reached sexual maturity (body mass of 30g) and begun cycling. The goal of this study was to evaluate the impact of environmental disturbances on stress and the relationship with the reproductive cycle. Preliminary analysis of faecal glucocortocoid metabolite (fGCM) levels (as determined by EIA) encompassing a period of two months prior to and following the move demonstrate a statistically significant peak (baseline + 2 SD) in fGCM levels (population average 94.0±33.5 ng/g) around the time of the move in 66% of the animals. Further analysis is underway to evaluate reproductive hormone profiles and possible effects from changing corticosterone levels.

PS16

Assessment of food-based enrichment in collared anteater (*Tamandua tetradactyla*) by non-invasive monitoring of adrenocortical activity

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Captive environments offer few opportunities for foraging or feeding and can negatively affects animal welfare. Measuring adrenocortical activity is a standard approach to evaluate stress and welfare in mammals. This study was conducted to: 1) physiologically validate an 11-oxoaetiocholanolone EIA, measuring faecal cortisol metabolites (FCM) with a 5ß-3α-ol-11-one structure to monitor adrenocortical activity in collared anteater faeces, and 2) investigate the influence of food-based enrichment on adrenocortical activity. Females (n=3) and males (n=2) were studied at Cordoba Zoo (Argentina). Faeces were individually collected (44 days) during pharmacological tests. The adrenal cortex was stimulated by ACTH (5 IU/kg, i.m., 10th day), then suppressed by dexamethasone (0.1 mg/kg, i.m., 37th day). After steroids were extracted

from samples, FCM were measured by EIA. Due to great individual variation in defecation frequency (2-8 faeces per week), measurements were individually pooled in pre-ACTH, post-ACTH, pre-dexa and post-dexa groups. Data were transformed to rank and non-parametric Friedman ANOVA was performed. Faecal cortisol metabolites levels were different among groups (post-ACTH> pre-ACTH and pre-dexa> post-dexa; $p<0.0001$). Three months later, adrenocortical activity response to enrichment was monitored (three faeces/week) during three 6-week periods. The first period was used as FCM baseline (control); then (2nd period) animals were daily subjected to food-based enrichment (i.e. ants, fruits, yogurt). Finally, regular feeding was again provided. A linear mixed statistical model was applied. No differences were detected on average (3.91±0.35, 3.44±0.22 and 4.51±0.35 µg/g, respectively). However, the number of FCM peaks/period was significantly reduced by enrichment ($p=0.02$, $F_{2,6}=7.79$). Peaks were defined on an individual basis as values that exceeded the control period mean plus 75th-percentile. Overall, results indicate that a non-invasive approach was validated to measure stress responses. This method has enormous potential for investigating different types of enrichment in this South American endemic xenarthra species.

PS17

Anthropogenic effects on European ground squirrels physiology

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Anthropogenic habitat alteration and its after-effects are a potential source of stress for European ground squirrel *(Spermophilus citellus)* populations, which increasingly have to cope with human-shaped areas throughout the species' range. To determine whether habitat alteration impacts the stress load of free-ranging populations in Austria, we live-trapped and faecal-sampled individuals during the active season both in a nearly unaltered steppe habitat (TD) and in a strongly altered Alfalfa meadow (FB). Overall and seasonal faecal cortisol metabolite (FCM) concentrations of sex and age cohorts were analysed and compared among the two study sites. FCM levels of adult (≥1 year) males and juveniles (<1 year) of both sexes were higher at FB than at TD, most pronouncedly when juveniles emerged in June. Adult females, however, showed neither site nor seasonal differences in FCM levels. Instead, they exhibited an increase in the simultaneously monitored faecal progesterone metabolites (FPM) at both sites during June. Our results indicate that at least in adult males and juveniles, stress load was affected by human activities. The altered vegetation led to highly abundant food at FB and, together with the isolation of the habitat, caused a high population density. Elevated FCM levels in both adult males and juveniles at FB coincided with highest individual density. This period is also known for increased predation pressure and peak levels of human recreational activities. The highest FCM levels were found in juveniles shortly after natal emergence at FB suggesting that this age cohort is most vulnerable to social stress, predation and human disturbances. Human habitat alteration at FB thus led to different levels of potential stressors. The clear lack of elevated stress load in adult females at FB may partly be related to the intriguing second oestrus cycle found in prior studies and supported by FPM data of the present study.

PS18

Body fat content and faecal cortisol secretion patterns in free-ranging juvenile common hamsters

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Free-ranging animals are often confronted with environmental or social challenges, activating the HPA axis. In hibernating small mammals a limited active season often causes additional temporal and energetic constraints. Adult females can produce up to three litters per season, therefore birth dates range from May to September resulting in different time spans available for early and late born offspring to grow and prepare for hibernation. These differences might lead to different tactics in the allocation of energy reserves for the winter and could affect

adrenal activity. Glucocorticoids are known to mediate hyperphagia during the fattening phase prior to hibernation in several species. Correspondingly these animals also showed elevated glucocorticoid levels late in the season, coinciding with excessive feeding and fat deposition. We investigated free-ranging common hamsters (*Cricetus cricetus*) in an urban area in Vienna. Glucocorticoid levels in live-trapped animals were measured non-invasively by analysing faecal cortisol metabolites (FCM). The proportion of body fat was calculated by integrating the morphometric parameters body mass, head, tibia, and foot length measured at each capture. In adult common hamsters FCM concentrations increased with intrasexual aggression in males and sexual interactions in females. We analysed FCM levels and the proportion of body fat in early- and late-born juveniles at natal emergence, after weaning, and shortly before onset of hibernation. Preliminary results showed that proportions of body fat were similar in both groups at natal emergence. Shortly before hibernation, however, early-born individuals had significantly more body fat than late-born ones. Furthermore, body fat proportions were positively correlated with FCM concentrations. These results indicate that early and late born juvenile hamsters use different strategies to prepare for the hibernation period. Early-borns appear to allocate body fat reserves, reflected in elevated FCM levels, while late-borns have to rely on food caches to survive over winter.

PS19

Preliminary investigation of the use of non-invasive measurements of faecal immuno-reactive corticosterone in rufous fantails (*Rhipidura rufifrons)*: A potential diagnostic tool for choice of best candidates for translocation

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Rufous fantails (*Rhipidura rufifrons*) are a small (<10g) insectivorous aerial foraging species native to the Commonwealth of the Northern Mariana Islands, with locally declining populations due to the invasive brown tree snake (*Boiga irregularis*). To preserve the species, the Mariana Avifauna Conservation Project is working to relocate wild-caught rufous fantails to other islands in the Marianas and to US zoological institutions to establish captive breeding populations. Historically, a decrease in body mass was used to indicate poor adaptation following collection. The purpose of this study was to test the feasibility of measuring faecal immuno-reactive corticosterone concentrations in rufous fantails as an additional diagnostic tool. Faecal samples from 17 wild-caught birds were collected during an eight day period prior to translocation. Each morning, accumulated samples were collected and immediately frozen. Due to small faecal mass, samples were pooled across two 4-day periods (P) for each bird. Pooled samples (wet weight: 0.27±0.10g) were extracted in 80% methanol and analysed using a corticosterone enzyme immunoassay (EIA; antisera CJM06, Munro, UC Davis, CA). Serial dilutions from pooled samples were parallel to the standard curve. Individual corticosterone concentrations ranged from 2.48 to 67.35 ng/g/pool. Concentrations from 13 of 17 birds decreased (-48.4±20.6%) and four increased (74.8±48.4%), however body weight increased in both groups from P1 to P2 (3.8±3.1% and 2.6±2.6%, respectively). This preliminary data suggests that faecal corticosterone values may compliment medical management, collection, and husbandry techniques, and act as an additional tool for choosing translocation candidates. Due to small mass, faecal output required pooling for corticosterone to be measured. Further studies are needed to confirm relationships between capture related stress and survivorship following translocation.

PS20

Motorways as stressors for wild wood mouse (*Apodemus sylvaticus*) populations

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Motorways represent one of the strongest anthropogenic impacts on natural areas producing several effects on mammal populations. Although they are recognized as a major cause of habitat fragmentation, habitat degradation and biodiversity loss, it is unknown whether motorways have other effects on mammal populations like an increase in physiological stress reactions. Faecal corticosterone metabolites (FCM) were analysed in wood mouse populations living along the AP-51 motorway (Spain). Wood mice were captured with Sherman live traps placed at differences distances (5 m, 500 m and 1000 m) from the motorway and checked every 8 to 10 hours. We collected fresh faecal samples from the trap for 424 individuals and measured concentrations of FCM by a 5α-pregnane-3ß,11ß,21-triol-20-one enzyme immunoassay. Trapped individuals were sexed and breeding status for females was determined by noticeable mammary development and/or a perforated vaginal membrane. Wood mice living close to the motorway had higher ($p<0.001$) levels of FCM (90±25 ng/g dry faeces) than those individuals captured further away (74±24 ng/g). Individual factors like sex and breeding condition also explained the variation found in FCM concentrations (females showed higher levels than males and breeding females showed an increase of FCM levels compared to the non-breeding ones). In addition, FCM correlated positively with weight of animals but not with vegetation variables (cover and height) and cattle presence. This study is the first one showing a significant impact of motorways' perturbation on wood mice physiological stress reactions. Understanding how free-living animals are influenced by human interventions could help to understand other subtle changes observed in wild animal populations.

PS21

Perception of sex pheromones in closely related mus species is modulated by glucocorticoids

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Olfactory cues play an important role in regulation of sexual behaviour in mammals. While the role of sex hormones in regulation of perception of chemical signals is well studied, the role of stress hormones remains quite unclear. Our earlier studies showed suppression of the response to receptive female odour of vomeronasal receptor neurons in males exposed to emotional /metabolic stress. The current study aimed to investigate the mechanisms of the modulatory effects of glucocorticoids (GCs) in perception of sex pheromones and the role of GCs in precopulatory reproductive isolation in closely related *Mus* species. Test subjects were two sympatric species: *M. musculus* (n=20), *M. spicilegus* (n=16) and laboratory form of *M. domesticus* (n=20). To monitor plasma testosterone and corticosterone we used ELISA technique. Faecal corticosterone metabolites were monitored non-invasively. To visualize activated neurons in vomeronasal organ (VNO) receptor tissue Fos-immunohistochemistry was used. To assess behaviour we utilized standard two and four preference tests. Male mice exposed to metabolic/emotional stress demonstrated no preference towards receptive female odour. These alterations in behaviour were accompanied by increased plasma corticosterone and faecal corticosterone metabolites. Pharmacological analysis showed that suppression of specific pattern of activation at the VNO receptor level in response to stimulation with receptive female odour correlated with plasma corticosterone and with faecal GC metabolites. We investigated the expression of steroid receptors (glucocorticoid, GCR, androgens, AR, and mineralocorticoid, MCR) in VNO receptor epithelium. A profound GCR-immunoreactivity in VNO receptor epithelium of male mice but not AR-immunoreactivity or MR-immunoreactivity was detected. Abundant expression of GCRs in VNO receptors suggests possible direct action of stress hormones on receptor cells. Acute and long-lasting exposure of *M. spicilegus* odours to commensal Mus species significantly elevated plasma corticosterone/faecal GC-metabolites and blocked testosterone response. Our data indicate that stress

may work as contributing factor in reproductive isolation of closely related Mus species.

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PS22

Non-invasive measurement of adrenocortical activity in blue-fronted parrot (*Amazona aestiva*)

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Parrots are frequently victims of exotic animal traffic, because of their ability to imitate human speech, sociability and beauty. It is estimated that from each 40 parrots trafficked, only one will survive due to poor welfare and stressful conditions during this process. The main glucocorticoid in birds is corticosterone and its quantification in the blood provides information about adrenocortical activity. However, in these small wild animals blood sampling is complicated, highly invasive and inappropriate to investigate stressful and welfare conditions. Thus, a non-invasive method is critically needed. The aim of this study was to physiologically validate an enzyme immunoassay (EIA) for the measurement of corticosterone metabolites (CM) in blue-fronted parrot (*Amazona aestiva*) droppings. We examined the effect of intramuscular injection of saline (0.2 mL), ACTH (25 IU) and dexamethasone (1 mg/kg) on CM in the droppings of 24 birds (12 of each sex; each bird received all treatments). Control birds remained undisturbed. Starting at the moment of the injections (8:00 AM) droppings were collected every 3 h for 24 h. CM were quantified using a cortisone EIA and analysed by repeated measure ANOVA. Control and saline groups showed the same pattern of variation of CM; in these groups concentrations started decreasing from 9 h onwards, reaching the lowest values 12 h (respectively, 106±74 to 130±90 ng/g faeces) and returned to initial values 21 h post injection. Following ACTH administration, CM concentrations started to rise from 3 h onwards, reaching peak concentrations from 6 to 9 h (1,529±1,119 and 2,562±2,322 ng/g faeces) and return to concentrations similar to the control and saline groups 21 h post injection. Following dexamethasone administration, CM concentrations started to rise from 3 h onwards (605±378 ng/g faeces), remained elevated until 6 h (475±207 ng/g faeces) and return to concentrations similar to the control group 12 h post injection. Our results demonstrate that a pharmacological stimulation of adrenocortical activity was accurately reflected by CM and that dexamethasone did not result in a distinct suppression of adrenocortical activity in blue-fronted parrots. By successful physiological validation, we demonstrated for the first time the suitability of an EIA to non-invasively monitor adrenocortical activity in blue-fronted parrot. This method opens up new perspectives for stress and welfare investigations in this frequently kept bird species.

PS23

Evaluation of the physiological stress response induced by winter sports in a black grouse (*Tetrao tetrix*) population from Lepontine Alps (VB)

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In the Alps, human outdoor leisure activities are an increasing conservation issue having a strong impact on wildlife, mainly on endangered species, such as black grouse (*Tetrao tetrix*). Since free-riding sports are one factor affecting this species, it is important to quantify the likely pressure of recreational disturbance on their health status considering that induced stress can alter animal fitness.
Using a non-invasive technique we examined the physiological stress response of a black grouse population from Central Alps induced by snow sports in relation to areas with different human-disturbance. During two winters (2011 and 2012) we sampled 58 fresh droppings from as many snow burrows to analyse concentrations of

corticosterone metabolites (CM) by an enzyme immunoassay (EIA). In 2011, faecal CM levels in high human-disturbance areas were significantly higher (mean: 512±107 ng/g) than those in moderate (151±42 ng/g) and low disturbed (138±42 ng/g) ones, moreover higher CM concentrations were observed in areas closer to ski lifts. On the contrary, in 2012, no significant differences in CM values were found between areas. Comparing results from sampling areas between study years, high-disturbed area CM levels were significantly higher in 2011 (m_{2011}: 512 ng/g; m_{2012}: 178 ng/g) while no significant differences were observed in moderate (m_{2011}: 151 ng/g; m_{2012}: 178 ng/g) and low (m_{2011}: 138 ng/g; m_{2012}: 211 ng/g) human-disturbed areas. Data show a great difference in high-disturbed areas CM levels between sampling years: in winter 2011 regular snowfalls have favoured the presence of skiers that elevate stress values and could represent a further threat to the fitness of black grouse. In 2012 the lack of snow has drastically reduced winter sports with a consequent lower human disturbance, reflected to CM levels. Moreover in 2012 the increase of population baseline CM values in low disturbed areas suggest a more stressful condition as they have to acclimate to these unusual meteorological trends.

PS24

Effects of mate separation on stress hormones, parasite load and immune parameters in free-living greylag geese

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Previous studies have shown that even short-term separation from social partners may increase glucocorticoid levels. Social interactions are not only potent regulators of the stress-response but also of immune function and health. To study the link between social factors, stress and health, we analysed the effect of mate separation in free-living, long-term monogamous Greylag geese (*Anser anser*). We caught and isolated the males of 8 pairs for 48 hours to examine the behavioural, adrenocortical, haematological and parasitological response to mate removal in the females and to social isolation in the males. During mate removal, the females showed behavioural changes, such as an increased number of distance calls, but surprisingly no elevated levels of corticosterone metabolites in the droppings. Their hematocrit decreased during mate removal, whereas leucocyte number and composition did not change. In contrast, the isolated males excreted elevated levels of corticosterone metabolites and showed a decrease in hematocrit as well as elevated leucocyte number and h/l ratio. In both sexes, the number of intestinal parasites was increased directly and one week after separation, but returned to baseline levels four weeks later. Although the effect in the isolated males was more pronounced, our experiment also indicated an immunosuppressive effect of mate removal in females in their natural social environment.

PS25

Physiological and behavioural response in carrion crows (*Corvus corone corone*) after relocation to a new environment

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Animals respond to unexpected environmental changes at both the physiological and the behavioural level. Within populations there are individual differences in the way they cope with such stressors. Long-term stressors might also affect an individuals' health negatively, for example by an increase in parasitic load, which could have negative consequences on the individuals' fitness. Therefore, especially in species living in complex social systems like carrion crows, we expect individuals to engage in affiliative behaviours in order to alleviate stress. In this study we investigated how a group of nine captive carrion crows responded to a severe stressor, i.e. a relocation of individuals to a new aviary. We examined physiological and behavioural responses using non-invasive techniques. We analysed two faecal samples per individual before and after relocation for determination of excreted corticosterone metabolite concentrations (CORT)

and excreted intestinal parasite products. CORT was analysed with a tetrahydrocorticosterone assay. We videotaped behaviour and tested individual differences applying personality tests, i.e. tonic immobility, open field test. Preliminary results indicate that excretion of coccidian oocysts significantly increased in the first week after relocation, returning to baseline levels afterwards. In contrast, excretion of Capillaria eggs did not change significantly. After relocation the behaviour 'body shaking' occurred more frequently, and the distance between individuals increased. Individuals with the highest increase in CORT after relocation also increased distance to the partner most. Duration of tonic immobility was negatively correlated with changes in CORT. Observed increases in excretion of parasite products may be indicative of the health effects of stressful event in carrion crows. In addition, the increased distance to partners was contrary to our expectation that they would engage in affiliative behaviour to alleviate stress. There was also significant variation in individual responses to the stressor, probably reflecting individual copying styles.

PS26

Vocal response to stressful situations in carrion crows (*Corvus corone corone*)

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Vocalisations are increasingly applied as a non-invasive method to assess stress in animals. Specifically, changes in rates and type of vocalisations, as well as acoustic measures within a signal can indicate stress responses. However, there are individual differences in the way animals deal with challenging and stressful situations. These personality differences might account for many observed behavioural variations. Here, we used a potentially stressful situation, the relocation into a new aviary, to investigate if vocalisations of carrion crows can be used as indicators of stress and how individuals' personality alters vocal stress response. We recorded vocalisations for six days before (120 min/day) and after (70 min/day) relocation, measuring call rates/minute, types of vocalisations emitted and acoustic parameters in the calls. In addition, we collected faecal samples before and after relocation to measure excreted corticosterone metabolites (CORT) as a control for a stress response independent of vocalisations. A subset of these samples was analysed via an enzyme immunoassay (tetrahydrocorticosterone: CMO BSA, raised in rabbits). Personality was assessed using tonic immobility and open field tests. After relocation, carrion crows showed a significant increase in the total number of calls/minute and in territorial caws/minute. Alarm cawing also increased. Acoustic analysis of territorial caws revealed significantly lower second formant frequencies after relocation. This parameter is known to decrease with higher arousal, indicating stress. Changes in excreted CORT before and after relocation did not correlate with changes in calling frequency. We further found a correlation between personality type and the number of calls/minute only before relocation, indicating that effects of personality onto vocalisations were less pronounced during stressful situations. Our results show that vocalisations in carrion crows are significantly influenced by stressful situations. Functionally, in carrion crows, signalling distress might be relevant for social interactions with other conspecifics, i.e. to avoid conflicts.

PS27

Does coping style affect preferences for feeding sites in ravens?

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Coping styles are defined as sets of correlated behavioural and physiological traits describing how animals cope with challenges. In general, individuals with proactive personalities are likely to be successful in constant or predictable conditions, whereas individuals with reactive personalities may cope relatively better with changing or unpredictable conditions. In our field site, a small valley in the northern Austrian Alps, non-breeding

ravens (seasonal range of n=40-100 out of a population of approximately 300 individuals) use a gamepark as predictable food source year round by scrounging from the zoo animals. Recent findings showed great variation among individuals in both the frequency of visiting the valley and utilizing the zoo animal feed. Here we address the question whether this observed variation can be linked to the personality concept. In a first step we assigned personality types to the wild ravens. After capture with a drop-in trap they were placed into a box for 1 h, droppings were collected and thereafter we allowed them to explore a novel environment (a room of 3x3x2.5 m) for 10 min. We videotaped and rated the ravens' exploration behaviour and determined the amount of excreted corticosterone metabolites (CM) in the droppings via an enzyme immunoassay against 11-oxoaetiocholanolone. The antibody was raised in rabbits against 5ß-androstane-3α-ol-11,17-dione-17-CMO:BSA. Despite the relatively small sample size (n=13) and high individual variation in CM we observed differences between individuals classified proactive and reactive on the basis of their behaviour in the novel environment. Proactive individuals tended to excrete lower amounts of CM during the isolation stress test compared to reactive copers. Until now (capturing and testing ravens is still ongoing) the coping style of an individual did not relate to its use of predictable food sources, which might be due to the relatively low sample size. In addition choice of feeding place might also be influenced by other factors (e.g. social interactions).

PS28

Plasma and salivary cortisol levels as the indicators of stress and fatigue during field exercise test in thoroughbred horses

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The aim of this study was to evaluate the use of saliva sampling for the measurement of cortisol concentrations detection of stress levels in young thoroughbred horses during race training. The group of 12 thoroughbred horses aged 3-5 years was examined during their speed training session at the Sluzeviec (Warsaw) racetrack. During the study, the horses galloped on the 1200 m sand track at a speed amounted to 14.4 – 15.3 m/s. Three saliva samples, and three blood samples were collected from each horse. Both types of samples were taken according to the following protocol: 1) at rest (marked as A), 2) immediately after return from the track (marked as B), 3) after 30 minutes restitution (marked as C). In each case, saliva was sampled firstly and then blood was taken. Blood lactic acid concentration (LA) was determined immediately using Dr Lange's enzymatic cuvette test. The concentrations of cortisol in saliva samples (CorS) were measured by enzyme-immunoassay method using the CORTISOL EIA kit DSL. For plasma cortisol (CorP) determination, the CORTISOL ELISA kit was used. Mean blood LA concentration increased after exercise to 13.4 mmol/l ($p<0.05$). CorP B was significantly higher than CorP A ($p<0.05$). Mean CorS A, B, and C did not differ significantly. The statistically significant coefficient correlations were found between blood LA B and CorP C ($r=0.60$, $p<0.05$); between LA B and CorS C ($r=0.60$, $p<0.05$); and between CorP C and Cor S C ($r=0.51$, $p<0.05$). The measuring of cortisol concentration in saliva samples taken from race horses 30 min after the end of exercise can be recommended to use in practice in field conditions to estimation the level of work-load and exercise-induced stress in race horses.

PS29

Relationship between social rank and adrenocortical activity in horses

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Social rank has previously been shown to be a predictor of glucocorticoid levels in both animals and humans. Social stress may be experienced by both high- and low-ranking individuals and varies as a function of social organisation and stability of the social hierarchy.

This experiment aimed to investigate the link between baseline adrenocortical activity, measured as faecal cortisol metabolites (FCM), and social rank in horses. Twenty-five Danish Warmblood geldings (2 or 3 years old) pastured in one group were included. Faeces were collected on four separate days where the horses had been undisturbed at pasture for 48 h. The first two collections were within the first month after the group was formed, whereas the last two collections were 2 and 3 months later, respectively. Social rank was determined through observations of social interactions on pasture in four limited resource tests (4 x 1 h) as well as during field observations (5 x 3 h), 2-3 months after group formation. The two first faeces collections had higher cortisol metabolite concentrations, compared to the two later collections (One way RM ANOVA: $F_{3,68}$= 7.85, p<0.001). There were significant correlations between almost all collection days, i.e. horses that had high FCM levels in one collection also tended to have high FCM levels on the other collection days. We found a strong correlation between the hierarchical order obtained from the limited resource tests and that obtained through field observations of social interactions (r_S=0.93, p<0.0001). No opposing displacements were observed, suggesting that the dominance hierarchy was stable two months after establishment of the group. There was a significant, negative correlation between FCM level and rank ratio (r_S= -0.43, p=0.035), indicating that higher ranking horses had lower FCM values. In conclusion, our results support the "subordination stress" hypothesis, i.e. that subordinates in a stable hierarchy experiences greater harassment and less control than dominants, which can lead to elevated glucocorticoid secretion.

PS30

Non-invasive monitoring of adrenocortical activity in captive fallow deer (*Dama dama L*)

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Analysis of glucocorticoid metabolites in faeces of various domestic and wild animals is an increasingly used non-invasive tool to monitor adrenocortical activity. Despite the fact that recent findings proved that glucocorticoid release can be triggered independent of stress, when combined with other parameters glucocorticoid levels still can provide significant information about aversiveness of certain events in animals' environment. To determine the excretion pattern for cortisol in fallow deer from extensive captive breeding in inland Croatia, we collected faecal samples during a one-year study at Radobojski Orehovec breeding site. Faecal cortisol metabolites were measured with an 11-oxoaetiocholanolone enzyme immunoassay, previously validated for fallow deer. Our study confirmed seasonal pattern of cortisol release as already seen in boreal deer species and free ranging fallow deer from the Brijuni Island. Highest concentrations of 11,17-dioxoandrostanes (a group of cortisol metabolites) were determined during the winter period (1044±424 ng/g faeces), followed by early summer (990±728 ng/g) and spring (791±645 ng/g). Significantly lower concentrations (when compared to winter and early summer) were determined during the late summer period (412±443 ng/g). Compared to concentrations in free-ranging fallow deer, levels of captive animals followed the same pattern, but with lower values for every season (significant difference only for summer period). This may be attributed to frequent contact and consequent adaptation to human presence in captive conditions (no room for long-distance avoidance) and less demanding food searching.

PS31

Effect of mild heat stress on faecal cortisol metabolites, blood minerals and energy metabolism of Holstein cows

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We investigated the effect of climatic conditions (daily average THI between 60 and 78) and access to shade on faecal cortisol levels, blood minerals and indicators of metabolic alterations in 20 Holstein cows. From June to September 2011,

the ILVO-herd was divided into a group with and without access to shade on pasture. Blood and faecal samples were collected from 10 matched shade-control pairs at respectively the evening and morning milking for 11 days with varying climatic conditions. Blood samples of control animals for the two days with lowest versus highest risk for heat stress were compared for indicators of metabolic alterations (glucose, alkaline phosphatase (ALP), triglycerides, cholesterol, creatinine, urea) and blood minerals (Na^+, K^+, Cl^-). All faecal samples were analysed for concentration of 11,17-DOA (a group of cortisol metabolites) to test whether it was associated with various climatic indices (e.g. temperature humidity index, THI) and whether it was affected by access to shade. The climatic conditions during the 11 days were relatively mild and not exceptional for summers in temperate regions. On the two days of highest heat stress risk (THI = 71 and 78) serum concentrations of ALP (P=0.046), cholesterol (P=0.011) and urea (P=0.004) were lower and the creatinine (P<0.001) and Cl- (P<0.001) concentration were higher than during the days of lowest risk for heat stress (THI = 60 and 64). Levels of 11,17-DOA were positively correlated with climatic indices such as the THI (p<0.001) but were not affected by access to shade. Shade-use was limited suggesting that conditions were unlikely to have induced severe heat stress. These results illustrate that blood serum ALP, cholesterol, creatinine, urea and Cl^- and faecal 11,17-DOA concentration are good indicators to evaluate mild heat stress in dairy cattle and – more importantly – potential preventative measures.

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PS32

Determination of stress in beef calves weaned using different methods

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Anti-suckling nose-clips (NC) and fence-line separation are methods of weaning calves gradually as opposed to abrupt separation. Crossbred beef cattle (n=192 cow-calf pairs) were used to evaluate three gradual weaning methods: "one-size fits all" NC (ONE), adjustable size NC (ADJ), and fence-line weaning (FL). In the control group (ABR), calves remained in pastures with their dams. On d -4 NC were placed on ONE and ADJ calves and FL calves were placed in pastures adjacent to their dams. Calves were completely separated from cows on d 0 and NC removed while ABR calves were abruptly weaned by remote separation. On d 0 a subset of the weaned calves (n= 96) were placed in dry lot pens where they were fed hay and a cotton by-product feed and observed for 4 days. Faecal samples were collected from calves on d -4, and d 0-4 and analysed for 11,17–dioxoandrostanes (11,17-DOA) using the 11-oxoaetiocholanolone EIA method. Data were analysed using the MIXED procedure of SAS (SAS Inst., Cary, NC, USA). Baseline measures of 11,17-DOA on d -4 did not differ between treatments (19.7±3.4 ng/g) and were similar to concentrations on d 4 (23.6±4.7 ng/g), with d -4 values differing from d 0 to 3 ($p \leq 0.02$). All calves had elevated 11,17-DOA on d 1 (60.7±6.4 ng/g). The gradually weaned calves' concentrations of 11,17-DOA decreased on d 2 while ABR remained elevated (70.1±6.7 ng/g) and differed from NC treatments ($p \leq 0.01$). By d 3 all treatments were similar (33.1±5.8 ng/g) and not different from d 4 values. ABR calves had an elevated level of stress for a longer duration than calves weaned gradually indicating that gradual weaning does reduce the stress of weaning.

PS33

Non-invasive measures of acute stress reactivity in European rabbit (*Oryctolagus cuniculus L.*) restocking fences and their relation to fitness

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The European rabbit is a key prey species in Mediterranean ecosystems that has undergone a dramatic decline in its natural ranges due to diseases and habitat loss. This situation has led to rabbit restocking actions, including translocations within enclosures where rabbits are acclimated to their new environment and start to breed, with the aim of further colonizing the surroundings. Stress is considered a key factor in animal translocation programmes because of the adverse effects of chronic stress. So far, glucocorticoid levels of wild rabbits, corresponding to basal and chronic stress conditions, have been studied in relation to predation level, predation perception and fitness. However, acute stress responsiveness and regulative capacity have not yet been tested in wild rabbits. In this study, acute stressors were applied to five rabbit restocking enclosures to mimic the most common sources of disturbance in the area: predation pressure, predator odour and human noise. Faecal samples were collected from 4-5 warren systems per enclosure. The first sample set was collected prior to the application of stressors to get baseline levels; the second set 12-24 hours later to quantify the reactivity to stressors; and the third set after 72 hours to account for the recovery. Faecal corticosterone metabolites were analysed by a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay, previously validated for wild rabbits. In the same enclosures, rabbit abundance was monthly estimated by pellet-counts. Enclosures and warren systems differed widely in rabbits' response and recovery after the stressors, with up to 3-fold increases in glucocorticoid levels. Results showed a straight-linear relationship between reactivity and recovery after short-term stressors and an inverse-U effect of the reactivity on long-term population growth. This finding suggests the importance of studying the responsiveness and recovery after stressors in the wild due to their potential influence on population growth.

PS34

Using non-invasive methods to determine animal stress in surgical training models

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Using animals for surgical training models can be stressful for animals involved; however, by optimizing the length of time for these procedures based on the stress hormone response, animal welfare can be improved. The aim of this project was to determine the effects of post-surgical procedure on stress and determine the minimum time that animal training model´s should experience this procedure. Twelve male New Zealand White Rabbits (*Oryctolagus cuniculus*) divided in three groups (48, 72 and 96 h), 4 rabbits each were used to reproduce a survival Bochdalek´s Congenital Diaphragmatic Hernia Model for Paediatric Surgery Training. The model was created performing a mini-laparotomy under general anaesthesia and a slit in the left diaphragm waiting 48, 72 and 96 h for presence of abdominal viscera in thorax and terminal surgical repair of diaphragmatic hernia. Faecal samples were collected from all rabbits for corticosterone metabolite (CM) analysis. The faecal collections were 24 h prior to and, 48, 72, and 96 h after surgery. CM concentrations were measured using a 5α-pregnane-3ß,11ß,21-triol-20-one enzyme immunoassay. All rabbits showed intestines into the thorax during thoracoscopic repair of the diaphragm defect. Prior to surgical procedures CM measures (mean, 248 ± 30 ng/g faeces) were lower ($p<0.01$) than all other time periods *(*48 h, 918±304 ng/g faeces; 72 h 1037±607 ng/g faeces; 96 h, 1178±415 ng/g faeces). Stress hormone numbers, while not statistically different, did increase with increasing time, and 48 h was the lowest post-surgical time point. And based on animal welfare, the 48 h group was the optimal group for this surgical model. This determination was made without holding them for stressful blood collection techniques to determine cortisol levels. Conclusion: CM measurement is a non-invasive tool to determine stress associated with a surgical procedure and helpful in surgical research.

PM1

Faecal cortisol metabolites to assess stress in wildlife: Evaluation of a field method on free ranging chamois

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Faecal cortisol metabolite (FCM) analysis is an established non-invasive tool to measure stress in captive and free ranging species. This method has great potential, but may be of limited suitability in field studies, where faecal samples from unknown individuals within a population are used for stress assessment. Besides individual and sex specific variation of glucocorticoid metabolites (GC), bacterial metabolism during storage may bias final results. Furthermore, FCM may not be evenly distributed in the faeces which could influence interpretation of measurements, when only small parts of the faecal mass are collected. We tested these assumptions on a population of free ranging alpine chamois (*R. r. rupicapra*) in the Salzkammergut, Austria. FCM were analysed with an established 11-oxoaetiocholanolone enzyme immunoassay measuring 11,17-dioxoandrostanes. In a storage experiment subsamples of five homogenized faecal samples were stored at 2 different temperatures for 1, 2, 4, 8, 16 and 24 h, respectively. No significant correlation between temperatures, length of storage and FCM levels was observed. To determine the within-sample variation, samples from four females and three males were divided into five respective parts, which were analysed separately. The mean coefficient of variation within these samples was <10%. Beyond these methodological issues, our sampling has shown a very high variation in FCM levels of chamois, even within individuals of the same sex in constant groups. Our results suggest that bacterial metabolism and uneven distribution of GC metabolites in the faeces may bias assay results, but are unlikely to cause the high individual variations. We report strong individual differences in FCM excretion to be the most likely reason for our results, which clearly constrains the use of FCM for stress assessment within a population of chamois, when samples are collected on an anonymous basis.

PM2

Validation of a faecal glucocorticoid assay and effects of daytime, sex and female reproductive state on glucocorticoid output in spider monkeys (*Ateles hybridus*)

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Brown spider monkeys (*Ateles hybridus*), endemic to Colombia and Venezuela, are critically endangered due to hunting and habitat destruction. Moreover, fitness and survival of extant populations may be compromised by increased stress resulting from living in fragmented forests. As a basis for examining the potential impact of these anthropogenic disturbances on physiological stress levels, we aimed to validate a method to assess glucocorticoid (GC) output from faeces. A second objective was to provide data on the potential influence of daytime, sex and female reproductive state on faecal GC concentrations. Using samples collected from five captive-housed spider monkeys during a capture-translocation event, we tested the ability of four glucocorticoid assays designed to measure cortisol, corticosterone, and groups of 5ß-reduced metabolites of cortisol (11-oxoaetiocholanolone and 11ß-hydroxyaetiocholanolone) to detect the expected increase in GC output due to the stressful event. Both group-specific cortisol metabolite assays showed high levels of immunoreactivity and a clear response to the stressor, while the two more specific assays showed much lower concentrations and were less responsive. Analysis of faecal samples (n=97) from wild-living males (n=5) and females (n=9) in the 11ß-hydroxyaetiocholanolone assay chosen as most suitable, demonstrated that GC concentrations were markedly affected by time of day and sex, with levels being 2.8 times higher in the morning than in the afternoon (p=0.007) and levels of males being twice as high as non-pregnant

females (p=0.03). Female reproductive state had no statistically significant effect on GC levels; however mean GC concentrations in pregnant and lactating females were 100% and 30% higher than those in non-pregnant animals, respectively. Our study provides the methodological basis for examining effects of habitat disturbance and fragmentation on the stress physiology of wild spider monkeys and demonstrates that daytime, sex and potentially female reproductive state need to be considered when interpreting GC output in this species.

PM3

Biological validation of enzyme immunoassays for measurement of faecal androgen and glucocorticoid metabolites in crested macaques (*Macaca nigra*)

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Crested macaques are endemic to the northern tip of Sulawesi and are currently critically endangered (IUCN Red List 2011). Non-invasive assessment of physiological function through faecal hormone analysis has not yet been established in this species. It is, however, essential to screen and validate existing assays before applying them to the species. The aim of our study thus was to find reliable enzyme immunoassays (EIAs) that could be used for non-invasive monitoring of androgen and glucocorticoid levels in crested macaques. For testing three different androgen enzyme immunoassays EIAs, epiandrosterone, testosterone and 5α-androstanolone, we compared results from a total of 43 faecal samples from five juvenile and five adult males. In addition, 99 samples from 6 individuals (males and females), which had undergone a 'stressful event', were used for testing three different glucocorticoid EIAs: cortisol, 11-oxoaetiocholanolone, and 11β-hydroxyaetiocholanolone. Furthermore, HPLC was conducted to investigate the specificity of the different antibodies and their ability to detect immunoreactivity associated with abundant androgen and glucocorticoid metabolites. The results show that with the exception of 5α-androstanolone, androgen EIAs were reliable in discriminating androgen levels between juvenile and adult males. Furthermore, HPLC data clarified that epiandrosterone is the most abundant androgen metabolite. On the other hand, both 11-oxoaetiocholanolone and 11β-hydroxyaetiocholanolone assays discriminated better between pre-/post-stress and stress periods than the cortisol assay. HPLC results showed here that the 11β-hydroxyaetiocholanolone assay detects the most abundant glucocorticoid metabolites. In conclusion, biological validation can be used to validate measurement of androgen and glucocorticoid metabolites in faecal samples of crested macaques, and it showed that epiandrosterone and 11β-hydroxyaetiocholanolone EIAs are the most reliable and useful assays to measure androgen and glucocorticoid metabolites in this species. Our results are important for the non-invasive monitoring of physiological function in crested macaques, and as such for conservation of the species.

PM4

A new quantification method for the analysis of non-metabolized faecal cortisol with a commercial ELISA Kit

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Cortisol, a glucocorticoid hormone of the hypothalamic-pituitary-adrenal axis, is one of the most investigated molecules in stress and animal welfare research. Sampling cortisol from faeces has the advantage of being non-invasive and, thus, stress-free for the animal. Hereby, effects of sampling procedure on cortisol concentrations such as by blood sampling can be prevented. An established method is to measure cortisol metabolites in faeces. In order to test whether it is also possible to measure the biologically active form of cortisol in faeces we established a new method based on a commercial ELISA Kit. Faecal

samples from 11 fattening bulls were extracted with methanol and water, dried and re-diluted in a kit-related solution. Samples were analysed both with a Luminescence-Immuno-Assay (LIA) and an Enzyme-Linked-Immuno-Sorbed-Assay (ELISA) kit (all IBL International) for human saliva cortisol. Additionally, 14 different samples from fattening bulls were sent to Fischer Analytics OHG (Bingen/Rhein) and analysed using liquid chromatography linked with tandem mass spectroscopy (LC/MS/MS).

Cortisol concentrations detected across LIA and ELISA ranged from 0.25 to 5.0 ng/g and were highly correlated (r^2=0.97). Cortisol concentrations from LC/MS/MS are in the same range.

Our results show that it is possible to analyse cortisol in cattle faeces and that commercial LIA and ELISA kits for the analysis of cortisol in human saliva seem to be suitable for this purpose.

PM5

Assay development for pregnancy testing of sitatunga (*Tragelaphus spekei*)

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Sitatunga are elusive swamp-dwelling African antelope whose populations have been described as declining. Therefore, the sitatunga population management plan has recommended growing the North American zoo population. Sitatunga are exceptionally flighty; most veterinary procedures are completed under anesthesia, but anesthetizing pregnant females can be risky. We aimed to identify which of two extraction protocols (80% methanol/shaking or 71% ether/freezing) and three commercially available antibodies (Sigma P1922 produced against progesterone-7, Sigma P5289 produced against progesterone-11α, or MP Biomedicals 07170016 produced against 11α-hydroxyprogesterone) would best detect a rise in faecal progesterone metabolites (PMs) during pregnancy using radioimmunoassay (RIA). At the Maryland Zoo in Baltimore, we collected twice-weekly faecal samples from three females across four pregnancies. The P5289 antibody bound to sitatunga faecal PMs only at very high concentrations and parallelism could not be obtained. Both P1922 and the 07170016 antibodies showed parallel dilutions of sitatunga faecal extracts and standards in using both extraction methods. However, comparison of five samples from each female in three pregnancy phases (pre-breeding, first-trimester, and second-trimester) revealed a significant main effect of pregnancy status with 07170016 ($F(2,36)=4.61$, $p=0.02$), but not P1922 ($p>0.18$). As a result, 07170016 was selected for the RIA. The methanol protocol (84±5%) more efficiently extracted tritiated progesterone added to faecal samples than the ether protocol (10±3%). However, RIA with ether extracts showed significantly higher PMs in pregnant than non-pregnant samples in three of four pregnancies ($U_{Joanie1}(87,24)=216$ $p<0.001$; $U_{Joanie2}(87,34)=426$ $p<0.001$; $U_{Pandora}(73,29)=749$ $p=0.02$; $U(58,47)=1276$, $p=0.57$), while RIA with methanol extracts did not ($p>0.08$ in three females, the fourth had significantly lower PMs during pregnancy). We conclude that ether extraction and the antibody 07170016 provide the best commercially available RIA for pregnancy diagnosis in sitatunga and discuss possible explanations for the assay system's failure to detect the fourth pregnancy.

PM6

Monitoring stress in captive and wild orangutans with cortisol measurements obtained from shed nest hair?

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The objective of this study was to assess whether the cortisol concentrations measured in orangutan hair are influenced by body part and the age of hair. This investigation is essential to validate the use of naturally shed hair as a marker for chronic stress. To date, the measurement of cortisol in ape hair is restricted to cut hair of defined body regions, which requires tame or narcotized animals. However, this is not applicable in non-invasive field studies where only shed hair deriving from unknown body parts can be found in nests. Using hair from up to 7 different body parts from 21 zoo orangutans, we found that the cortisol concentration in hair is not

dependent on the body region from which the hair derived. The potential influence of the age of the hair on cortisol concentration, for example due to loss of cortisol over time, was tested by measuring cortisol concentration in 3 cm segments along hair shafts. There was no significant difference between proximal and distal segments of 15 cm long hair strands. Even along 42 cm hair shafts, collected from an individual that was kept under constant conditions, the cortisol concentrations in the segments did not differ considerably. Interestingly, in other individuals we could correlate several distinct changes in cortisol concentration along the hair shaft with stress events in the life history. Depending on hair length, we could correlate cortisol changes with events up to 4 years ago, suggesting the potential of hair as retrospective stress calendar. We conclude that naturally shed hair can be used to assess the basal stress level of orangutans and most likely other great apes in the field.

PM7

Levels of cortisol in hair samples of non-human primates

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Short-term changes in glucocorticoid levels are relatively easily assessed in biological fluids, e.g. serum and urine. Values from such samples are specific for a single (or short) time-point and can be highly variable. Recently, methods were developed to determine cortisol levels in hair. Cortisol in the circulation is incorporated into the hair shaft during growing and reflects the average cortisol level over the growth period of the hair. This makes hair suited to determine chronic hormone levels. We analysed the hormone levels in hair samples of a colony of 150 long-tailed macaques that were moved from their original location to a new location at the BPRC. Hair samples were taken before, during and after moving over a total follow-up period of more than 2 years. In addition, we sampled our resident colony of rhesus macaques and common marmosets to be able to determine the basal level of hair cortisol in these colonies and to compare the different species. Our results show cortisol levels in the hair samples increased due to moving the long-tailed macaques to a new area. Two months after settling in at the new location, cortisol levels showed a significant decline. To our surprise, the levels of hair cortisol in the new housing situation were significantly decreased compared to pre-move levels, possibly due to improved housing facilities. Rhesus and long-tailed macaques at the BPRC showed comparable levels of cortisol in hair samples. In contrast, cortisol levels in hair from marmosets were much higher. In addition, very young animals from the three species tested showed extremely high cortisol levels. Our conclusion is that hair samples of primates can be used to determine cortisol levels reflecting prolonged stress levels. This can be used to study improvement and monitoring of welfare of animals under captive conditions.

PM8

A preliminary investigation for monitoring reproductive status using prostaglandin F2-alpha metabolite (PGFM) and proposed pregnancy biomarker ceruloplasmin in the urine of giant pandas

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Following ovulation, non-pregnant giant pandas experience pseudo-pregnancy where behavioural, physiological, and hormonal changes are indistinguishable from those observed for pregnant females; hence, the investigation for alternative markers of pregnancy. The objectives of this study were to: 1) evaluate whether urinary metabolites of prostaglandin F2alpha (PGFM) are a useful panda pregnancy marker; 2) compare two different methods of measuring urinary ceruloplasmin activity (CP), a new biomarker of pregnancy; and 3) relate the PGFM and CP profiles to urinary progestagen metabolites. PGFM was measured in serial urine samples collected from a pregnant (n=1) and pseudo-pregnant (n=1) panda using a PGFM EIA (K022-H1, Arbor Assays). CP was measured using either Arbor Assays (AACP) endpoint colorimetric assay (K035-H1) or Memphis Zoo (MZCP) kinetic CP assay. During the luteal phase

PGFM increased 3-5 times above basal levels in both the pregnant and pseudo-pregnant animals shortly after the secondary rise in progestagens and decreased similar to progestagens as the luteal phase ended. This data suggests that PGFM may not be a good biomarker of pregnancy but might be a valuable marker for parturition. Both the endpoint and kinetic CP activity assays produced similar urinary profiles over time. There were fewer CP activity peaks during the luteal phase in the pseudo-pregnant compared to pregnant profile. Prior to the secondary rise in progesterone, CP activity (Units/mg Cr), as measured by both methods, was lower during pseudo-pregnancy (Mean±standard error, MZCP: 10.5±1.5; AACP: 964±147) than pregnancy (MZCP: 39.8±6.9; AACP: 1410±239) supporting the use of CP as an early pregnancy biomarker using frequent serial urine collections. Analysis of samples from additional oestrous cycles of confirmed pregnant and non-bred females are underway. PGFM and CP changes may help elucidate aspects of pregnancy, implantation, pregnancy loss, and parturition in giant pandas.

PM9

Endocrine disruption associated to oral administration of atrazine in European quail (*Coturnix coturnix coturnix*)

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Atrazine (ATZ) has been excluded from the registration process of pesticides in the EU. Despite that, it is still one of the most commonly used herbicides in the world. It has been reported to exhibit reproductive toxicity in rats, fish and amphibians. However, only slightly toxic effects to birds have been described. The present study investigates the toxicity of ATZ in European quail (*Coturnix coturnix coturnix*) and its ability to influence endocrine control of reproduction in sexually mature females. ATZ was orally administered as a single dose of 25 or 100 mg/kg to female European quail on days 0, 5 and 10 of the experiment. The animals were sampled at days 15, 30 and 45. To evaluate possible oestrogenic effect of ATZ, hepatic oestrogen receptor α (ERα) gene expression, and plasma 17β-estradiol (E_2) and vitellogenin (Vtg) levels were measured. Data were statistically analyzed by means of Kruskal-Wallis test followed by Dunn's post hoc test with $P<0.05$. ATZ significantly ($p=0.016$) increased the expression of hepatic ERα at both doses. Simultaneously, an increase in plasma E_2 concentrations was also observed reaching values of 23.22 pg/ml, 29.40 pg/ml in plasma from quails receiving doses 1 and 2 respectively (E2 plasma level in control quails was 16.97 pg/ml). ATZ at 100 mg/kg increased the circulating concentration of Vtg (84.9, 634.9 and 486.5 ng Vtg/ml plasma in control, dose 1 and dose 2, respectively) but this effect was not related with an increase in hepatic Vtg mRNA levels. These results led to the conclusion that ATZ could be causing deleterious effects in avian species provoking an increase of ERα expression and E2 plasma levels, which could modify plasma Vtg levels. However, this mechanism should be explored and the causality of these findings established.

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PM10

Effect of brute propolis on the diet of *Callithrix jacchus* used in research: Maintenance of the body weight

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The species *Callithrix jacchus* has been used as an experimental model in several research studies involving endocrine function, pharmacology, behaviour and nutrition. Due to its small body size and adaptability to captivity, this species is often used as a biological model. It is therefore indispensable that adequate nutritional support is provided to ensure improved reproduction and well-being of animals in research and/or conservation centers.

Propolis has been shown to improve the immune response, digestive disorders and feed conversion in animals. The goal of this study was to evaluate the effect of propolis on the weight maintenance of twelve couples of marmosets (*Callithrix jacchus*) kept in captivity before and after mating. Twelve adult pairs were kept in natural conditions of temperature, humidity and light-dark cycle. There were a pre-experimental period of acclimation and the use of operational conditioning with positive reinforcement for weighing. The control group received only routine diet, and the treatment group received a routine diet added of a measuring spoon of "petit Suisse" cheese (Danoninho®) to improve the palatability, and 2% of brute propolis powder, in relation to the daily consumption estimated by individual once per week. The administration of brute propolis in the feed of *Callithrix jacchus* helped males and females gain weight before reproduction and helped to maintain the weight of the treatment group (compared to control group) during the gestational period of the females.

The experiment was performed with authorization by the Animal Ethics Committee (Comissão de Ética no Uso de Animais – CEUA) of the Universidade Estadual Paulista 'Júlio de Mesquita Filho', authorization no. 126/2010. We are thankful to the São Paulo Research Foundation (FAPESP) for the financial support (2009/52654-9; 2010/51926-2).

Last name	First name	Pres.	Category	No.
Adachi	Itsuki	Repro	Poster	14
Agil	Mohammad	Methods	Poster	3
Ahrendt	Line	Stress	Poster	29
Amrein	Martin	Stress	Talk	3
Armstrong	Diana	Stress	Poster	1
Bashaw	Meredith	Methods	Poster	5
Behringer	Verena	Miscell	Talk	9
Boland	Holly	Stress	Poster	32
Brenner	Michaela	Stress	Poster	17
Brown	Janine	Repro	Talk	1
Bryant	Jocelyn	Repro	Talk	7
Busso	Juan	Stress	Poster	16
Cáceres	Sara	Stress	Poster	7
Carlitz	Esther	Methods	Poster	6
Chelini	Marie-Odile	Repro	Poster	19
Cho	Katherine	Repro	Poster	7
Cinque	Carlo	Stress	Poster	4
Clapp	Jim	Stress	Talk	7
Cortinovis	Luana	New Ave	Talk	3
De la Casa	Irene	Methods	Poster	9
Dehnhard	Martin	New Ave	Talk	1
Deschner	Tobias	Methods	Talk	2
Edwards	Katie	Repro	Poster	3
Edwards	Katie	Methods	Talk	4
Eggermann	Julia	Stress	Poster	8
Ellis	Tim	New Ave	Talk	2
Engelhardt	Antje	Miscell	Talk	5
Fanson	Kerry	Stress	Poster	12
Ferraz	Myrna	Methods	Poster	10
Ferreira	Joao	Stress	Poster	22
Formenti	Nicoletta	Stress	Poster	23
Formigaro	Costanza	Repro	Poster	23
Freeman	Elizabeth	Stress	Poster	14
Fureix	Carole	Stress	Talk	6
Ganswindt	Andre	Miscell	Talk	7
Gholib	Assahad	Stress	Poster	3
Girard-Buttoz	Cédric	New Ave	Talk	5
Glenk	Lisa Maria	Stress	Talk	8
Graham	Katie	Stress	Poster	19
Guimarães	Marcelo	Repro	Poster	12
Hart	Russ	Methods	Poster	8
Haymerle	Agnes	Methods	Poster	1
Heistermann	Michael	Repro	Talk	2
Hirschenhauser	Katherina	Miscell	Talk	1
Hofman	Linda	Methods	Poster	7
Kalliokoski	Otto	Methods	Talk	5
Kędzierski	Witold	Stress	Poster	28
Keeley	Tamara	Repro	Talk	4
Kersey	David	New Ave	Talk	4
Kneidinger	Nadja	Stress	Poster	11
Konjević	Dean	Stress	Poster	30
Kouba	Andy	Methods	Talk	8
Lepschy	Michael	Methods	Talk	3

Last name	First name	Pres.	Category	No.
Loretto	Matthias-Claudio	Stress	Poster	27
Ludwig	Sonja	Stress	Poster	24
MacKinnon	Katherine	Repro	Poster	10
Martínez	Leticia	Stress	Poster	6
Metrione	Lara	Stress	Talk	5
Micheletti	Tatiana	Repro	Talk	5
Millesi	Eva	Repro	Poster	21
Moscovice	Liza	New Ave	Talk	6
Möstl	Erich	Methods	Talk	1
Munerato	Marina	Stress	Talk	4
Navarro	Alvaro	Stress	Poster	20
Nemeth	Mathias	Miscell	Talk	10
Neumann	Christof	Stress	Poster	2
Painer	Johanna	Repro	Poster	15
Paris	Monique	Repro	Poster	13
Pavlova	Ekaterina	Repro	Talk	6
Petow	Stefanie	Methods	Poster	4
Pfeiffenberger	Ulrike	Repro	Poster	5
Pimm	Robyn	Stress	Poster	15
Ponzio	Marina	Repro	Poster	20
Rehnus	Maik	Stress	Poster	13
Rettenbacher	Sophie	Methods	Talk	6
Riechert	Juliane	Methods	Talk	7
Rimbach	Rebecca	Methods	Poster	2
Rivera	Ramon	Stress	Poster	34
Rösner	Sascha	Miscell	Talk	3
Ruiz	Leire	Stress	Poster	33
Sanderson	Jennifer	Miscell	Talk	8
Santymire	Rachel	Miscell	Talk	4
Schwarzenberger	Franz	Repro	Poster	2
Siutz	Carina	Stress	Poster	18
Spreafico	Michela	Stress	Poster	25
Steinman	Karen	Repro	Poster	17
Stöwe	Mareike	Miscell	Talk	2
Szipl	Georgine	Stress	Poster	26
Tiddi	Barbara	Repro	Poster	11
Touma	Chadi	Stress	Talk	2
Trotter	Jess	Repro	Poster	4
van der Goot	Annemieke	Repro	Poster	1
Van der Weyde	Leanne	Stress	Poster	9
Van laer	Eva	Stress	Poster	31
Viau	Priscila	Repro	Poster	9
Vick	Mandi	Repro	Poster	18
Vlamings	Bart	Stress	Poster	10
Voznessenskaya	Vera	Stress	Poster	21
Walker	Susan	Repro	Poster	6
Wallner	Bernard	Miscell	Talk	6
Wheaton	Catherine	Repro	Talk	3
Wheeler	Brandon	Stress	Poster	5
Wielebnowski	Nadja	Stress	Talk	1
Zaccaroni	Annalisa	Repro	Poster	22
Zanetti	Eveline	Repro	Poster	8
Zezza	Letitia	Repro	Poster	16