

PROCEEDINGS

of the 7th ISWE CONFERENCE

13-16 October 2019 – Kruger National
Park, South Africa



Hosted by the Mammal Research
Institute of the University of Pretoria
and South African National Parks



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Welcome to the 7th Conference of the International Society of Wildlife Endocrinology!

Hello (English); Sanibonani (isiZulu); Molo (isiXhosa); Hallo (Afrikaans); Thobela (Sepedi); Dumela (Setswana); Lumela (Sesotho); Abusheni (Xitsonga); Sanibona (SiSwati); Avuwani (Tshivenda); Salibonani (isiNdebele) and welcome to the 7th Conference of the International Society of Wildlife Endocrinology, held this year for the first time on African soil at Nombolo Mdhuli Conference Centre, Skukuza - Kruger National Park, South Africa from 13-16 October, 2019.

We have had a productive and partly challenging time since our last conference and were working hard to improve our role as a supportive communication platform for wildlife endocrinologist worldwide; thereby trying to upscale the perception and public image of our Society. Our endeavours included the successful resurrection of our continuously updated website (www.ISWE-endo.org), an increased presence on social media platforms, and the establishment of an ISWE Newsletter amongst others. We would like to thank our former board members Mandi Schook, Ned Place, David Kersey, Elizabeth Freeman, Marina Ponzio, and Gaby Mastromonaco for all the hard and productive work during their term, you really set a high standard. Consequently, we welcomed Katie Edwards, Annie Newell-Fugate, Grace Fuller, Diana Koester, and Jocelyn Bryant, as our new members on the board, and I must admit that they just continuing to strive towards excellence.

This year, 103 colleagues from 18 different countries registered for our conference, resulting in a total of 39 accepted oral and 46 poster presentations, and we would like to thank the 27 colleagues reviewing abstracts this year. Our intended first ever trainee-mentor luncheon attracted 32 trainees, and we received 33 very competitive submissions for student travel awards. We were able to award two travel scholarships to assist student researchers with expenses for attending the conference and we would like to use the opportunity to thank conference sponsors Arbor Assays for putting forward US\$1000 and Enzo Life Sciences for sponsoring US\$2500. Further we would like to thank the National Research Foundation (NRF) of South Africa, for supporting our conference with a respective grant of US\$6600.

We are very excited to have our two keynote speakers, Prof Nigel Bennett from the University of Pretoria, elaborating on the endocrine correlates of socially-induced infertility in eusocial mole-rats; and Prof Ann MacLarnon from Durham University, recapturing the explanatory power of wildlife endocrinology to inform from mechanism to even evolutionary constructs. Further we are delighted that Prof Rupert Palme from the University of Veterinary Medicine in Vienna agreed to lead a workshop around the advances and problems related to the non-invasive measurement of glucocorticoids. Finally, we thank all the presenters, for sharing new techniques and approaches and providing new insight into the exciting field of wildlife endocrinology.

A very special thanks goes to our Conference Host, SAN Parks, for allowing us to have our conference in such a unique setting and for participating in the development of the additional activities program, which will hopefully help to make this event a very special one. Further we would like to give a big hand of applause to Jackey Deacon from Mpumalanga Promotion Events, Karin Fischer from the Mammal Research Institute, and our Conference Chair, Diana Koester, who have worked tirelessly behind the scenes for many months to organize what we hope will be a productive and entertaining conference for all and an experience of a lifetime for at least some.

Finally, we thank you all for attending the conference. It is through these conferences that we learn how to better study and protect our wildlife. We are a hugely collaborative group, and thus we encourage you all to engage in stimulating discussions during the conference and welcome new members into our unique scientific family.

We hope you all enjoy your stay in South Africa.

Andre Ganswindt, ISWE Chair

ACKNOWLEDGMENTS

We express our sincere thanks to the following colleagues for reviewing abstracts:

Janine Brown	Smithsonian Conservation Biology Institute, Front Royal, VA, USA
Martin Dehnhard	Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
Elizabeth Freeman	New Century College, George Mason University, Fairfax, VA, USA
Andre Ganswindt	University of Pretoria, Pretoria, South Africa
Katarina Jewgenow	Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
Rachel Santymire	Lincoln Park Zoo, Chicago, IL, USA
Karen Steinman	SeaWorld & Busch Gardens Reprod. Research Ctr, San Diego, CA, USA
Mandi Wilder Schook	Disney's Animals, Walt Disney World, FL, USA
Anneke Moresco	Denver Zoo, Denver, CO, USA
Natalia Prado-Oviedo	Smithsonian Conservation Biology Institute, Front Royal, VA, USA
Grace Fuller	Detroit Zoological Society, Royal Oak, MI, USA
Ashley Franklin	Saint Louis Zoo, St. Louis, MO, USA
Katie Edwards	Chester Zoo, Chester, UK
Franz Schwarzenberger	Dept. of Biomedical Sciences, Univ. Veterinary Medicine, Vienna, Austria
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Juan Manuel Busso	Institute of Biological and Technological Research, Cordoba, Argentina
Marina Ponzio	National University of Cordoba, Cordoba, Argentina
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Jocelyn Bryant	Brookfield Zoo, Brookfield, IL, USA
Lara Metrione	South-East Zoo Alliance for Reproduction & Conservation, FL, USA
Nei Moreira	Federal of Parana University (UFPR), Parana, Brazil
Jella Wauters	University of Ghent, Belgium
Ratna Ghosal	Ahmedabad University, India
Sanjeeta Pokharel	Centre for Ecological Science, Indian Institute of Science, India
Michael Heistermann	Leibniz Institute of Primate Research, Germany
Megan Brown	Smithsonian Conservation Biology Institute, Front Royal, VA, USA
Tamara Keeley	University of Queensland, Queensland, Australia

Our Conference Planning Subcommittee

Andre Ganswindt (Host)
Diana Koester (Conference Chair)
Marina Ponzio
Lara Metrione
Jocelyn Bryant
Grace Fuller
Shana Lavin
Katie L Edwards
Breanne Murray
Mandi Schook
Jella Wauters

We warmly thank our invited speakers for contributing keynote lectures:

Professor Ann MacLarnon - Department of Anthropology, Durham University, United Kingdom.
FROM MECHANISM TO EVOLUTION: THE EXPLANATORY POWER OF WILDLIFE ENDOCRINOLOGY.

Professor Nigel Bennett - Professor of Zoology, University of Pretoria, South Africa. TEASING APART SOCIALLY-INDUCED INFERTILITY IN NON-REPRODUCTIVE FEMALE AND MALE EUSOCIAL MOLE-RATS IN HARSH ARID ENVIRONMENTS.

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PLENARY SPEAKER: PROFESSOR ANN MACLARNON



Ann MacLarnon is a Professor of Evolutionary Anthropology in the Department of Anthropology at Durham University, United Kingdom. Professor MacLarnon has worked on a broad range of areas in evolutionary anthropology, including comparative studies of life histories, spinal cord, brain size and gut size, and the evolution of human speech and breathing control. In recent years, her main focus has been on ecological physiology. Professor MacLarnon has directed a non-invasive hormone lab for fifteen years, which she recently moved to Durham. She has collaborated with many behavioral ecologists working on primates and other mammals to investigate together questions of reproductive, stress, and energetic ecology combining physiological and behavioral measures.

PLENARY SPEAKER: PROFESSOR NIGEL C. BENNETT

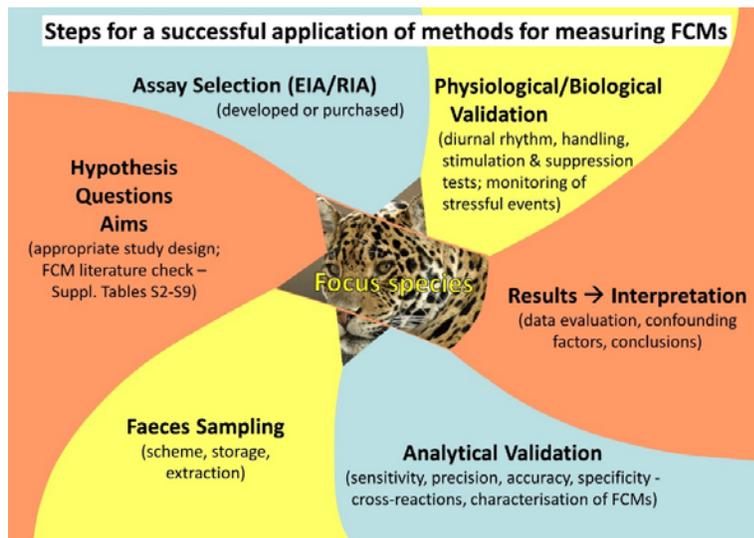
Nigel C. Bennett is a Professor of Zoology at the University of Pretoria (UP) and occupies the Department of Science and Technology/National Research Foundation (NRF) Research Chair in the field of Mammalian Behavioural Ecology and Physiology and the UP Austin Roberts Chair of African Mammalogy. Professor Bennett's research focus is on ecology, animal physiology and behavior using the African mole-rat as his model animal. He and his colleagues have investigated the ecological and physiological factors that affect the control of reproduction and the evolution of sociality. Professor Bennett's research has set the benchmark for our understanding of phylogenetic and ecological constraints regulating reproductive success and social evolution in mammalian species.



WORKSHOP: DR. RUPERT PALME

NON-INVASIVE MEASUREMENT OF GLUCOCORTICOIDS: ADVANCES AND PROBLEMS.

Glucocorticoids (GCs) are a central component of the neuroendocrine stress response and their measurement is frequently used to evaluate the impact of potentially challenging situations. Their faecal metabolites are often measured as a non-invasive evaluation of adrenocortical activity in wildlife species. When applied properly, such methods are a powerful tool to a variety of disciplines. However, appropriate study design, methodology, validations, and interpretation are all key to obtaining meaningful data. This interactive workshop



will address some pitfalls and potentially confounding factors, and provide an insight into the steps researchers should take before embarking on such techniques, especially focusing on validation and interpretation of results.

Rupert Palme is Associate Professor in the Division of Physiology, Pathophysiology and Experimental Endocrinology at the University of Veterinary Medicine, Vienna. His work focuses on the metabolism and excretion of steroid hormones and the development and application of non-invasive methods for their

quantification in various animal species. He and his coworkers succeeded in establishing the world-wide first non-invasive method for stress-assessment in animals by measuring faecal glucocorticoid metabolites. Their assays are now applied successfully in an increasing number of bird and mammalian species in a variety of research fields.

Coralie Munro Memorial Travel Scholarship: Justine Hudson
M.S. Student, Department of Biological Sciences
University of Manitoba



Justine Hudson's research aims to use endocrine techniques to understand how climate change and anthropogenic activity will impact the health of marine mammals in the Canadian Arctic. Justine spent 2 summers in Churchill, Manitoba collecting snot samples from free-swimming beluga whales to determine stress levels in the Western Hudson Bay beluga population. While on dry-land, she can be found in the lab measuring hormones along baleen plates to better understand the relationship between foraging activity and stress in bowhead whales. Aside from her research experience, Justine is also a passionate science communicator whose research has been highlighted in CBC, National Geographic, and Up Here Magazine.

ISWE Travel Scholarship: Laura McCaw
M.S. Student, Department of Ecology & Evolutionary Biology
University of Toronto Scarborough



Laura McCaw's research is part of a 45-year study in Canada's boreal forest investigating the cyclic fluctuations of snowshoe hare populations. Her research aims to elucidate how natural stressors, such as fluctuating predation risk and food availability, act from the individual level up to the population level over generations. Laura measures glucocorticoids via fecal enzyme immunoassay, blood plasma radioimmunoassay, and hair cortisol analysis as indices of stress. In addition, her research assesses changes in gene expression of steroid hormone receptors in newborn snowshoe hare, through maternal epigenetic programming, such as DNA methylation. She hopes that her work can help provide a basis for understanding the molecular mechanisms behind population decline in both cyclic and non-cyclic populations experiencing chronic and acute stressors in the wild.

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Maternal effects in snowshoe hare: impact of predation risk on offspring

Laura K McCaw¹, Sophia G Lavergne¹, Rudy Boonstra¹

¹ University of Toronto, Ontario, Canada

Presentation type: Oral Presentation

Presenting author: Laura McCaw

Corresponding author email: laurakmccaw@gmail.com

Abstract:

In the vast boreal forest of North America, the keystone herbivore - snowshoe hare (*Lepus americanus*) experiences 8-11 year population cycles primarily driven by their predators (lynx, coyotes, great-horned owls). High predation risk triggers the decline phase of the cycle causing both direct mortality and indirect chronic stress effects that curtail reproduction and survival. The decline phase is followed by the 2-5 year low phase, when the population fails to recover in spite of available and abundant high-quality forage and relative absence of predators. This suggests population-level programming of these chronic stress effects across generations. During pregnancy, maternally-derived stress hormones can act to program the physiology, morphology and behaviour of offspring, thus affecting their function and fitness throughout life. Through a natural monitoring study spanning the hare cycle, we investigated the impact of maternal stress on offspring hippocampal-hypothalamic-pituitary-adrenal axis function and regulation. Specifically, we looked at changes in the expression of steroid hormone receptors (glucocorticoid and mineralocorticoid) in key brain regions. This allowed us to investigate the role of prenatal epigenetic programming and elucidate the upstream mechanisms that shape an individual's present and future responses to a stressful environment. We found coordinated changes in gene expression involved in the stress response that varied by phase of the cycle and resulted in fitness consequences to offspring and grand-offspring. We hypothesize that these changes to the stress axis take time to dilute out of the population before population growth can begin again. This research is foundational in demonstrating how a key natural stressor (varying intensity of predation risk) impacts a wild population as a whole through intergenerational programming. In addition, our study provides a basis towards understanding the molecular mechanisms behind well documented chronic stress effects seen in a variety of wild species at both the individual and population levels.

**Using dermal cortisol to measure the stress physiology of wild and captive Wyoming toads
(*Anaxyrus baxteri*)**

Rachel M Santymire¹, Allison B Sacerdote-Velat², Andrew Gygli³, Doug A Keinath³, Sheila Poo⁴, Kristin M Hinkson⁴, Elizabeth M McKeag³

¹ Lincoln Park Zoo, IL, USA; ² The Chicago Academy of Sciences, IL, USA; ³ US Fish & Wildlife Service, WY, USA; ⁴ Memphis Zoo, TN, USA

Presentation type: Oral Presentation

Presenting author: Rachel Santymire

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Abstract:

Amphibian populations are declining worldwide and increased exposure to environmental stressors, including diseases and pathogens, including *Batrachochytrium dendrobatidis* (*Bd*), are proposed drivers of decline. Our goal was to use a novel, noninvasive dermal swabbing method to measure glucocorticoids and investigate the relationship among disease, environmental conditions and stress physiology in the critically endangered Wyoming toad. Our objectives were to validate the use of dermal swabs to measure cortisol (via enzyme immunoassay using Munro R4680) using an adrenocorticotropic hormone (ACTH) challenge on 8 captive Wyoming toads (4 ACTH: 2M, 2F and 4 saline as a control: 2M, 2F); 2) compare fecal glucocorticoid metabolites (FGMs) pre- and post-ACTH experiment; and 3) investigate dermal cortisol across three reintroduction sites with varying population success and disease prevalence. In females, saline resulted in two elevated swabs at 0 min (2.2-fold) and 60 mins (13.3-fold) and ACTH resulted in 2 (4.6-fold at 0 min and 3.0-fold at 90 min) and 3 elevated swabs (10.7-fold at 0 min, 3.4-fold at 30 min and 3.9-fold at 60 min). In males, saline resulted in 1 elevated swab (8.6-fold) at 0 mins. For males, ACTH resulted in elevated swabs at 45 mins (4.9 fold) and 60 mins (3.1 fold) post-injection, respectively. Saline did not elicit a change in FGMs post-injection; however, the male had elevated (7.6-fold) 2 days post-ACTH and female had elevated (1.6-fold) FGMs on day-4 post-ACTH. Dermal cortisol was similar across reintroduction sites; however, wild males at the Mortenson site had higher ($P=0.002$) swab cortisol in August compared to June. Wild *Bd+* toads (752.8 ± 68.0 pg/ml swab) had higher ($P=0.045$) swab cortisol than *Bd-* toads (500.0 ± 95.3 pg/ml swab). Dermal hormonal analysis is a novel tool that can be used to study amphibian stress physiology and can provide information on how environmental conditions are impacting population success.

Non-invasive monitoring of glucocorticoid metabolite concentrations in urine and faeces of the sungazer

Juan Scheun^{1,2}, Dominique Greeff¹, Andre Ganswindt^{2,1}

¹ National Zoological Garden, SANBI, Gauteng, South Africa; ² Mammal Research Institution, Department of Zoology and Entomology, University of Pretoria, Gauteng, South Africa

Presentation type: Oral Presentation

Presenting author: Juan Scheun

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Abstract:

Developing non-invasive techniques for monitoring physiological stress responses has been conducted in a number of mammal and bird species, revolutionizing field-based endocrinology and conservation practices. Despite this, very few studies have monitored and validated glucocorticoid concentrations of reptiles and amphibians. Furthermore, the correlation between glucocorticoid metabolite concentrations excreted in the urine (uGCM) and faeces (fGCM) of reptiles, which are excreted in unison, has yet to be explored. The aim of the study was to validate a method for monitoring uGCM and fGCM concentrations of the endangered cordylid lizard, the Sungazer (*Smaug giganteus*). A previously conducted biological validation, using faecal samples, was unsuccessful. As such, an adrenocorticotrophic hormone (ACTH) challenge was conducted on one male and two females (F1, F2), with both urine and faecal material being collected during baseline and post-injection periods. Steroid extracts were analysed with four enzyme immunoassays (EIAs) namely; 11-oxoetiocholanolone, 5 α -pregnane-3 β -11 β -21-triol-20-one, tetrahydrocorticosterone, and corticosterone. For fGCMs, both the 5 α -pregnane-3 β -11 β -21-triol-20-one (554 \pm 290.6%) and tetrahydrocorticosterone (481 \pm 57.6%) showed a considerable response following ACTH administration in all study subjects. Similarly, for uGCMs, the 5 α -pregnane-3 β -11 β -21-triol-20-one (299.7 \pm 77.5%) and tetrahydrocorticosterone (517.9 \pm 155.2%) again showed the highest response in all study animals. As such, both EIAs appear to be suitable for monitoring glucocorticoid metabolite concentration in Sungazer urine and faeces. Clear sex-specific differences were also found in the time to peak fGCM (M: 105h, F1: 24h, F2: 24h) and uGCM (M: 97h, F1&F2: 27h) concentrations. Furthermore, both EIAs showed a significantly higher concentration of glucocorticoid metabolites in faeces compared to urine for both sexes. Collectively, the findings of this study confirmed that both urine and faeces can be used to non-invasively assess adrenocortical function in *S. giganteus*.

Validation of a non-invasive technique using faecal glucocorticoid metabolites to measure stress in leopards (*Panthera pardus*)

Andrea B Webster^{1,2}, Richard EJ Burroughs^{3,4}, Peter Laver^{1,5}, Andre Ganswindt^{1,2}

¹ Endocrine Research Laboratory, Department of Anatomy and Physiology, University of Pretoria, Pretoria, South Africa; ² Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa; ³ Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa; ⁴ Centre of Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa; ⁵ Animal Demography Unit, Department of Biological Sciences, University of Cape Town, Cape Town, South Africa

Presentation type: Oral Presentation

Presenting author: Andrea Webster

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Abstract:

The majority of suitable leopard habitat lies outside of protected area boundaries. Leopards utilizing areas under different anthropogenic influences may therefore be exposed to various environmental, physiological and psychosocial stressors. A reliable test system that facilitates the non-invasive monitoring of physiological responses to stress in free-ranging leopards exposed to different anthropogenic influences has not yet been established. Five different enzyme-immunoassays (EIA's) were evaluated for monitoring adrenocortical function in the leopard based on faecal glucocorticoid metabolite (fGCM) analysis. After performing an adrenocorticotrophic hormone (ACTH) stimulation test, evaluating the influence of different feeding regimes on gastrointestinal transit time (GIT) and investigating the stability of fGCM post-defecation, faecal samples from free-ranging leopards roaming in a peri-urban and a conservation area were collected for comparison. Our results indicate that a 5α -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA is most suitable for assessing adrenocortical function in male and female leopards. Peak fGCM concentrations post ACTH increased from baseline ($0.39 \mu\text{g/g DW}$ and $0.29 \mu\text{g/g DW}$) by $>300\%$ and $>200\%$ in males and females respectively. Although not statistically significant, GIT was longer in females (40.8 hours) than in males (31.2 hours) and appears to be influenced by food availability, quality and quantity. Post-defecation fGCM concentrations remained stable for up to 6 days. Overall median fGCM concentrations were higher in free-ranging females (median: $2.22 \mu\text{g/g DW}$; range: $0.04 - 6.09 \mu\text{g/g DW}$) than in males (median: $0.63 \mu\text{g/g DW}$; range: $0.06 - 1.79 \mu\text{g/g DW}$), which may be linked to reproductive status in females. The ability to non-invasively and reliably assess adrenocortical function in this cryptic African predator can assist with local wildlife management, human-wildlife conflict and conservation issues.

Effects abnormal prolactin secretion has on the metabolic health of acyclic female African elephants (*Loxodonta africana*)

Matthew D Krcmarik^{1,2}, Janine L Brown¹, Larry Rockwood², Lance Liotta², Natalia Prado¹

¹ Smithsonian Conservation Biology Institute, Virginia, USA; ² George Mason University, Virginia, USA

Presentation type: Oral Presentation

Presenting author: Matthew Krcmarik

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Abstract:

Reproductive success is an increasing concern for captive African elephants (*Loxodonta africana*) in North America. Chronic hyperprolactinemia (HPRL), elevated prolactin secretion, has been proven to have a strong association with ovarian dysfunction in African female elephants. Previous studies have identified metabolic effects correlated with acyclicity, such as higher concentrations of insulin and leptin and lower glucose-to-insulin (G: I) ratios. However, metabolic effects from chronic elevated prolactin have yet to be investigated in elephants. In humans, hyperprolactinemic women have shown to have increased risks for accelerated atherosclerosis, hyperandrogenemia, decreased metabolism, and insulin resistance. The aim for the study was to explore possible associations between abnormal prolactin secretion (high and low) and metabolic disorders in elephants. One year of serum samples, collected biweekly, were obtained from African female elephants (n=36) with varying status of prolactin secretion (high= 12, normal= 12, low= 12). Biomarkers were assessed to compare thyroid function, glucose and lipid metabolism and cardiovascular health amongst the high, normal, and low prolactin state groups. Generalized linear mixed models (GLMs) were performed in R. Results determined that high prolactin secretion is associated with abnormal TSH and thyroid hormone production, elevated cortisol and cholesterol, and reduced fructosamine. Low prolactin individuals were found to have heightened levels of testosterone. Taken together, this study highlights several areas in need of further study to further advance our understanding of African elephant physiology and the etiology of hyperprolactinemia in female elephants.

An effect of multiple paternity on females' and kittens' immune and hormonal status in felids

Sergey V Naidenko¹, Galina S Alekseeva¹, Polina S Klyuchnikova¹, Mariya N Erofeeva¹

¹ A.N.Severtsov Institute of Ecology and Evolution, Moscow, Russia

Presentation type: Oral Presentation

Presenting author: Sergei Naidenko

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Abstract:

All felid species display promiscuity as the most common mating strategy, which results in multiple paternity. Carrying of embryos sired by different males may differ from single-male pregnancy because of higher immune stimulation by diverse antigens. The aim of this study was to estimate the immune and hormonal status of females having single/multiple-male pregnancy and their offspring. This study was conducted at the experimental station Tchernogolovka. The Eurasian lynx (litter size and body mass of cubs) and domestic cats (all experiments) were the subjects of the study. Females mated with one-three males during the mating season. We monitored several parameters: litter size, changes in body mass of females and kittens up to the age of one month, hormonal status of females during pregnancy (hair and serum cortisol and progesterone) and newborn kittens (hair cortisol), immune status of females and newborn kittens (leukocytes number, IgG concentration, hemoagglutination). Paternity was determined through molecular-genetic methods. Both Eurasian lynx and domestic cats gave birth to larger litters after mating with two-three males. There were no differences in body mass of newborn kittens and their growth rate between litters sired by one/two males. We did not find differences in hormonal status of females carrying litters of one/two males either. The hair cortisol was similar in kittens from the litters sired by one/two males. However, the females with multiple paternity litters had higher level of leukocytes during the pregnancy. The IgG concentrations and hemoagglutination test showed slightly higher results for these females as well. We found some differences in immune (but not the hormonal) status of females that carried litters with single/multiple paternity. It is likely that supposed higher genetic variability of males' antigens after multi-males fertilization results in stimulation of immune system of the mothers. This study was supported by Russian Science Foundation № 18-14-00200.

Assessment of fecal progesterone and estrogen metabolites for reproductive monitoring in female aye-ayes (*Daubentonia madagascariensis*).

Elizabeth M Donelan¹, Stephanie Schuler¹, Matt Miller², Anneke Moresco³, Erin Ehmke⁴,
Ronald Evan, Terri L Roth¹

¹ Cincinnati Zoo and Botanical Garden, Center for Conservation and Research of Endangered Wildlife, Ohio, USA; ² Cincinnati Zoo and Botanical Garden, Center for Conservation and Research of Endangered Wildlife, Ohio, USA; ³ Denver Zoo, Colorado, USA
⁴ Duke Lemur Center, North Carolina, USA

Presentation type: Oral Presentation

Presenting author: Elizabeth Donelan

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Abstract:

Little is known about the female aye-aye's (*Daubentonia madagascariensis*) reproductive physiology. Although females display overt estrual cues including vulvar swelling, and increased vocalizations, efforts to assess reproductive activity by hormone metabolite analysis have been inconclusive. In this study, we set out to determine if: 1) it is possible to extract reproductive hormones from aye-aye feces, 2) fluctuations in those hormones follow the cyclical pattern of vulvar swelling, 3) ovulation can be confirmed by an increase in progesterone metabolites, and 4) pregnancy can be diagnosed. Samples were collected 3-5 times per week from three females for 8 weeks, 31 weeks, and 2 years, over the course of the study. Samples were dried, and hormone metabolites extracted via shaking in 5 mL 90% ethanol. Extracts were dried down and reconstituted in 1 mL of 90% ethanol, then diluted 1:50 in assay buffer before analysis. Progesterone (P4) metabolites were measured with the Arbor Assays P4 mini-kit, and estradiol (E2) metabolites were measured using the R0008 antibody (Coralie Munro, UC Davis) and the Arbor Assays double antibody competitive EIA method. Serially diluted, pooled samples were used to validate both E2 and P4 assays based on parallelism with the standard curve ($r = 0.9958$ and 0.9967 , respectively). Peaks in the E2 profiles coincided with peaks in the vulvar swelling. Elevations in P4 were not observed following estrus even if breeding activity was observed. However, P4 did increase significantly during the second half of pregnancy (2744.5 ng/g) and dropped sharply after parturition (531.8 ng/g). In conclusion, it is possible to extract estrogen and progesterone metabolites from aye-aye feces, and fecal hormone metabolite monitoring does have potential as a tool for monitoring reproductive activity in aye-ayes. Most importantly, fecal progesterone analysis can be used to diagnose pregnancy in this species from mid to late gestation.

The clockwork whale: annual testosterone cycles reconstructed from baleen of adult male bowhead whales (*Balaena mysticetus*)

Kathleen E. Hunt¹, C. Loren Buck¹, Justine M. Hudson², Alejandro Fernández-Ajó¹, Mads P. Heide-Jørgensen³, Steven H. Ferguson⁴, Cory J.D. Matthews⁴

¹ Northern Arizona University, Arizona, USA; ² University of Manitoba, Manitoba, Canada; ³ Greenland Institute of Natural Resources, Greenland; ⁴ Fisheries and Oceans Canada, Manitoba, Canada

Presentation type: Oral Presentation

Presenting author: Kathleen Hunt

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Abstract:

Reproductive cycles of mysticete (baleen) whales are poorly understood, due to a lack of methods for obtaining endocrine samples. In male whales, patterns of gonadal activity are almost entirely obscure. We recently demonstrated that baleen contains steroid hormones that are deposited as it grows, such that a piece of baleen can be used to reconstruct the endocrine history of the whale over the timespan of baleen growth, 1-2 decades in bowhead whales (*Balaena mysticetus*). To investigate occurrence and potential cyclicity of seasonal testosterone elevations in male bowheads, we analyzed baleen from nine adult males from eastern Canada and Greenland. Baleen specimens of 184-314 cm in length were drilled at 2 cm intervals (each interval representing ~1-2 mo), producing powder from which testosterone (T) was extracted with methanol and assayed via enzyme immunoassay. Baleen of all nine males contained evenly spaced T peaks strongly suggestive of annual cycling. Mean distance between adjacent T peaks ranged from 14 cm in the largest whales to 19-21 cm in smaller whales, significantly correlated with individual annual baleen growth rate estimated from stable isotope data, and agreeing with prior findings of slowed baleen growth in larger individuals. Change in T peak amplitude over time was significantly related to whale body length (a proxy of age), with T peaks increasing over time in smaller individuals and decreasing over time in larger individuals. The smallest male had no T peaks in the first half of his baleen, but began cycling in the second half. A case was noted of a "skipped" breeding season (a double-length gap in otherwise regularly spaced peaks). We conclude that male bowhead whales exhibit annual testosterone cycles, and that baleen hormone analysis enables examination of reproductive seasonality, onset of sexual maturity, potential reproductive senescence, and other aspects of reproduction in male mysticetes.

Relationship between thyroid and testosterone hormones in aquarium and wild sand tiger sharks (*Carcharias taurus*)

Cayman L Adams¹, Jennifer T Wyffels¹, Linda M Penfold¹

¹ South-East Zoo Alliance for Reproduction & Conservation, Florida, USA

Presentation type: Oral Presentation

Presenting author: Cayman Adams

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Abstract:

Thyroid hormones (TH) exhibit a variety of functional roles and their structure is highly conserved across animal taxa, but the role of TH in elasmobranchs is poorly understood. In fish, there is speculation that they are important for the onset of seasonal reproduction, and decreased TH has been linked with reduced sperm numbers, decreased sperm viability and impaired follicle development. This study examined plasma TH, testosterone and iodine concentrations in aquarium and wild sand tiger sharks. Plasma samples collected from aquarium sharks (n=26) and wild sharks (n=32) were analyzed for triiodothyronine (T3) and thyroxine (T4) using Arbor Assays kits (cat#K056 and cat#K050). For T3, serial dilutions of untreated plasma demonstrated parallelism to the standard curve, and percent recovery of known amounts of hormone was 115% ($R^2=0.9991$) in females and 102% ($R^2=0.9995$) for males. For T4, plasma was ether extracted to obtain parallelism with the standard curve, and percent recovery of known amounts of hormone was 99% ($R^2=0.9994$) in females, and 98% ($R^2=0.9994$) for males. Blood plasma iodine analyses were completed by MSU Diagnostic Center using an inductively coupled plasma mass spectrometer. Hormone and iodine concentrations were compared between aquarium and wild sharks. Aquarium sharks had no difference in T4 compared to wild sharks ($P>0.05$), higher iodine ($P<0.05$) and lower T3 and testosterone concentrations ($P<0.05$). Lower T3 in aquarium sharks might be the result of a failure to convert T4 to the active T3. Iodine was not correlated to TH ($R^2=-0.21$). Based on these findings, it is possible that the reproductive issues seen in aquarium sand tiger sharks might be associated with thyroid dysfunction. Further investigation into resolving differences in TH between wild and aquarium sharks could result in better nutrition management that promotes successful reproduction in sharks under managed care and has potential implications for other *ex situ* elasmobranch species.

Social context mediates testosterone's effect on snort acoustics in male hyrax songs

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Presentation type: Oral Presentation

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Abstract:

Testosterone affects physical and motivational states, both of which have a major impact on vocalization structure and acoustics. Rock hyrax (*Procavia capensis*) males advertise their quality by production of a complex call (i.e., a song) composed of repetitive elements. The snort, a low frequency, noisy element, encodes information on the singer's age and social rank via harshness, as measured by jitter (i.e., waveform frequency stability) and length. We have previously theorized that the snort carries a double message, advertising concomitantly both vocal stability and aggression. Our past findings revealed that testosterone levels are related to both vocal elements and social status of male hyraxes, suggesting that hormonal mechanisms may mediate the motivation for aggressive and courtship behaviors. Here we examined whether long-term androgen levels are related to snort acoustics and song structure, by comparing levels of testosterone extracted from male hyrax hair, with acoustic and structural parameters. We found that songs performed by individuals with higher testosterone levels include more singing bouts and longer, smoother snorts, but only in those songs induced by external triggers. It is possible that hyraxes with higher levels of testosterone possess the ability to perform higher-quality singing, but only invest the effort in situations of high social arousal and potential benefit. Surprisingly, in spontaneous songs, hyraxes with high testosterone were found to snort more harshly than low-testosterone males. This suggests that the aggressive emotional arousal associated with testosterone is naturally reflected in the jittery hyrax snort, but that it can be masked by high-quality performance.

Hand raised, pack-living dogs' and wolves' urinary oxytocin levels following interactions with human partners

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Presentation type: Oral Presentation

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Abstract:

Several domestication hypotheses suggest that dogs can establish special bonds with humans, and involvement of the oxytocin (OT) system was proposed. OT levels of both pet dogs and captive wolves have been studied in a social interaction test before, but no study compared dogs and wolves raised and kept under comparable conditions. To address this, we investigated if human-animal-interactions (HAI) correlate with urinary OT levels in hand raised, pack-living dogs and wolves with similar human experiences. We predicted more interactions and greater OT increase following HAI in dogs than wolves. Wolves were predicted to prefer the closer bonded partner and show higher OT levels with the bonded than the familiar humans. Ten wolves and eleven dogs were tested 1) with a bonded, 2) a familiar human, 3) in a baseline condition, and 4) after food delivery without physical contact. Urine samples were collected within 60-90 minutes after testing and analysed using a validated Enzyme Immunoassay. To test whether OT levels were influenced by condition, species, sex, or behaviour (body contact and stress signals), we used linear mixed models into which we included these predictors as fixed effects and subject as random effect. To control for the effect of feeding and breeding season, these factors were included as well. Behaviourally both species showed a preference for the bonded over the familiar human ($P < 0.0001$). In contrast to the social conditions, "food only" led to the greatest OT increase ($P = 0.02$). No effect of species ($P = 0.062$) but an effect of sex ($P = 0.03$) emerged. No effects of body contact ($P = 0.14$) or stress signals ($p = 0.93$) on OT levels were found for either species, although dogs showed more stress signals than wolves ($P = 0.01$). Our results suggest that, while both species prefer to interact with the bonded partner, no corresponding rise in OT is evident following social interactions.

Urinary creatinine concentrations reveal that wild chimpanzee mothers positively influence muscle mass development years past weaning

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Presentation type: Oral Presentation

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Abstract:

In animals with altricial offspring, most post-natal growth occurs during immaturity. Allocation of resources towards growth may involve depletion of resources for other physical systems. However, maternal care and provisioning during immaturity may optimize offspring growth patterns. The prolonged juvenile dependence and maternal care seen in humans is a distinctive life history adaptation and is likely adaptively selected to enable sustained somatic and brain development. In chimpanzees, immature individuals continue to associate with their mothers for years beyond weaning, however, whether this prolonged association influences growth patterns and represents a similar life history adaptation with humans is not clear. Creatinine, a parameter often used as a correction factor for urine concentration when measuring urinary hormone levels, is a by-product of cellular metabolic activity occurring in muscle tissue. Urinary excretion levels of creatinine correlate with muscle mass and, therefore, concentrations of urinary creatinine (once corrected for specific gravity and thus dilution of a sample) can be used as a non-invasive measure of muscle mass in wild chimpanzees. We examined how maternal presence and characteristics influence the development of muscle mass in wild chimpanzees from the Taï population in Ivory Coast aged 0-15 years. The dataset included repeated sampling of 86 individuals, resulting in 1577 urinary creatinine measures. Maternal presence positively influenced offspring muscle mass such that orphans had significantly less muscle mass than non-orphans (P -value = 0.019). For offspring with mothers, those with top-ranking mothers had greater muscle mass (P = 0.027). Male offspring with greater levels of maternal investment (length of inter-birth interval length prior to birth of siblings) also had greater muscle mass (P = 0.033). Our results show that chimpanzee mothers have an extended influence on offspring phenotypes and illustrate the broader potential of urinary creatinine in the monitoring of early life history strategies in wildlife.

Drivers of population performance: physiological responses to environmental stress in equids (Grevy's zebra, *Equus Grevyi*, Cape mountain zebra, *E. zebra zebra* and Carneddau Welsh mountain ponies *E. ferus caballus*)

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Presentation type: Oral Presentation

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Abstract:

Human activity is changing the world at an unprecedented rate. Physiological biomarkers can provide insight into how animals respond to environmental change, predict long-term impacts of environmental change and interpret the consequences of conservation interventions. Physiological biomarkers, however, remain virtually untapped as tools for understanding large-scale macro-ecological and evolutionary processes of population dynamics. Here we use non-invasive faecal biomarkers to assess physiological responses in wild equid populations to address large-scale conservation questions. First, we discuss a previous study on population differences in faecal glucocorticoids and androgens in the Cape mountain zebra, *Equus zebra zebra*. Faecal glucocorticoid concentrations were elevated in individuals from seasonal low-quality habitats and faecal androgen concentrations were higher in populations with a male biased sex-ratio. Furthermore, population growth rates were negatively associated with faecal androgens, indicating a possible relationship between hormone profiles and fitness. However, we have since reassessed the validity of different assays for a population of free-ranging ponies that suggest a more complex interpretation of these assays than we originally assumed. We are currently extending this approach to evaluate spatial and temporal responses of the endangered Grevy's zebra, *Equus grevyi*, to environmental change. By using a combination of steroid stress and reproduction hormones, along with measures of oxidative stress, we assess variation in population performance of Grevy's zebra across a habitat gradient. This framework allows us to provide cross-population evidence for how population dynamics in these wild equid species can be linked to individual physiological biomarkers. These techniques can be applied to other species (both *ex situ* and *in situ*) to investigate whether conservation interventions are effective and assess causes of variation in population performance. Furthermore, they can provide data for species distribution models in order to help predict future responses to environmental change, and ultimately the long-term viability of the species.

Perioovulatory changes in behaviour and faecal oestrogen and progesterone metabolite concentrations could predict the fertile period in female African wild dogs (*Lycaon pictus*)

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Presentation type: Oral Presentation

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Abstract:

Artificial insemination (AI) can aid African wild dog conservation, but determining the female's fertile period is difficult because methods used to time AI in domestic dogs are not feasible without immobilization in wild canids. Therefore, we explored whether certain behaviours and faecal oestrogen (fEM) and progesterone (fPM) metabolite concentrations could predict the fertile period in late oestrus. Behaviour was observed in 3 alpha females up to 6 days before until 6 days after the mating period, and frequently collected faeces were analysed for fEM and fPM concentrations using enzyme-immunoassays. To link behaviour and faecal steroid levels with oestrus and ovulation, females were immobilized 2-3 times during the perioovulatory period to evaluate vulvar condition and blood steroid hormones, and to perform vaginal cytology, vaginoscopy, and ovarian ultrasound. Late oestrus could be distinguished from pro-oestrus, early oestrus and dioestrus using behaviour, with a 2- to 5-fold higher rate of male-female affiliative behaviour, sexual follow, alpha male initiating behaviour, ride-up and copulation ($P \leq 0.05$). Sexual behaviours, and male-female resting declined significantly or ceased the day after last mating. One female was anovulatory with no increase in fPM/plasma progesterone concentrations during oestrus/dioestrus. The two other females showed a 2.5- to 3-fold increase in fPM concentrations in late oestrus compared to pro-oestrus values ($P \leq 0.05$). Elevated fEM concentrations that rose during pro-oestrus, declined to baseline levels by late oestrus. Vaginal cytology and vaginoscopy could not discriminate late from early oestrus or pro-oestrus. Ultrasonography could not adequately distinguish between follicles and CL. In summary, an increase in distinct behaviours during late oestrus coupled with rising fPM and declining fEM concentrations appear to be suitable parameters to determine the fertile period in African wild dogs. However, data show that, without frequent invasive sampling, blood steroid hormone concentrations, vaginal cytology, vaginoscopy and ultrasonography can be misleading.

Ecological Espionage: application of non-invasive methods to evaluate anthropogenic disturbance in elusive African clawless otter (*Aonyx capensis*)

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Presentation type: Oral Presentation

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Abstract:

The usefulness for the application of non-invasive methods such as measuring physiological stress in faecal samples specifically applies to cryptic animals such as the African clawless otter (*Aonyx capensis*). These methods are feedback free and therefore do not reflect on the animals' physiology or alter its behaviour. Increased applicability might be given by examining animal adaptation to man-made environments in a time of increasing environmental change caused by anthropogenic disturbance. We aimed to compare faecal glucocorticoid metabolites (fGCM) concentrations as a measure of stress and group size and time of activity revealed by camera trap data in African clawless otters occurring in a peri-urban area and two natural areas. From the five enzyme immunoassays (EIA) tested, a cortisol EIA was the most conservative for measuring fGCMs in African clawless otters and fGCM concentrations remain comparable until three hours post defaecation. The fGCM concentrations for otters from the peri-urban area ($n = 20$; $0.468 \pm 0.539 \mu\text{g/g}$ dry weight (DW)) were significantly ($P = 0.019$) higher compared to the natural areas ($n = 20$; $0.245 \pm 0.219 \mu\text{g/g}$ DW). We found a significant difference ($P = 0.007$) between group sizes in the peri-urban area ($n = 112$; range = 1 – 5) and natural areas ($n = 29$; range = 1 – 3). There was a significant difference in otter activity time between the natural areas and peri-urban area ($p = 0.039$), the peri-urban otters were more nocturnal (91% of records at night) compared to natural otters (81%). These results suggest that African clawless otters display physiological and behavioural responses to adapt to man-made environments. This is the first study to apply the combination of faecal sample analysis and camera trap data on African clawless otters.

Non-invasive measurement of reproductive and stress hormone metabolites in Asian elephants: an approach towards conservation

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Presentation type: Oral Presentation

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Abstract:

About 60% of world's Asian elephants, spread across 13 Asian countries, are native to India. Because of varied ecological disturbances, we require multi-pronged conservation strategies and an understanding of demography, reproductive and stress status of elephants. We have been comprehensively addressing them. One such measure is to assess reproductive and stress status of free-ranging Asian elephants in ecologically-sensitive zones of India. Due to legal, ethical and operational reasons, we adopted a non-invasive sampling of animals' fresh dung samples, developed process for their storage and transportation. We employed specific enzyme linked immune-sorbent assays (ELISAs) for estimating hormone (fecal) metabolites for progesterone (allopregnanolone: f5-alpha-P-3OH: fPM), testosterone (epiandrosterone: fAM) and glucocorticoids (11-oxo-aetiocholanolone: fGCM). We addressed three issues: (1) assessment of estrous cyclicity and estrous behaviour (fPM), (2) musth status of males (fAM and fGCM) and (3) stress status of crop-raiding males and/or females (fGCM). Field sites chosen were the dry and moist tropical forests of Mudumalai, Bandipur, Nagarhole and Kaziranga National Parks. The estimated hormone metabolites' contents (mg/g dry fecal sample) were in the range: 0.5-10 for fPM; 0.24-15.3 for fAM and 0.32-11.0 for fGCM. The three respective values significantly ($P < 0.05$; t-test or LME model) correlated with the examined three physiological states i.e., estrous, musth and stress status. We demonstrated, for the first time in Asian elephants, that measurements of fecal steroid hormone metabolites help assess reproductive and stress status of animals with significant correlative measures to various ecological, anthropogenic and stress factors, experienced by elephants. Our efforts on endocrinological assessments, by non-invasive strategies, to monitor reproductive and stress physiology and health of free-ranging Asian elephants are crucial in the conservation and have applications in term of reducing anthropogenic stress-response in Asian elephants, inhabiting within the protected and/or human-encroached habitats. (Funding: Dept of Environment and Forests, Govt of India & Indian Institute of Science, Bangalore).

Climate change and human disturbance increase stress levels in a wild ungulate.

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Presentation type: Oral Presentation

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Abstract:

Anthropogenic disturbances now significantly impact nearly every habitat on Earth, and rapid human-induced environmental changes are forcing many species to adapt at an unprecedented pace. In East Africa, climate change is predicted to severely alter weather patterns and result in reductions of forage quality and availability. Furthermore, exceedingly pervasive human land use and land cover change, mainly due to agricultural expansion, is significantly changing and reducing the region's natural habitat. This study tested the hypothesis that the combined effects of climatic conditions, through forage quality, and anthropogenic land use would significantly influence fecal stress hormone (glucocorticoid metabolites) levels in wild impala. We employed a space-for-time approach using remotely sensed data on normalized difference vegetation index (NDVI) as a measure for forage availability, combined with spatially explicit proxies of human disturbance across areas of different protection management strategies, within the Greater Serengeti Ecosystem in Tanzania. Impala stress levels increased significantly with decreasing NDVI (i.e., low food availability). Furthermore, NDVI was by far the most important predictor for impala stress levels, explaining as much as 20% of the variation in stress levels. Stress levels significantly increased with human settlement density. Additionally, impala stress levels were higher closer to the national park border, potentially indicating increased poaching intensities. These results underpin the importance of forage availability in predicting stress levels in a wild ungulate. Forage availability is predicted to become increasingly variable due to climate and land use change. Ungulates with more specific diet or habitat preferences than impala may be more affected by future climate and land use change in the region. Understanding how animals respond to and cope with changes in forage quality and human land use across different protected areas is important for conservationists and managers to better protect species at risk and predict population viability.

Snot for Science: a non-invasive technique for measuring cortisol in free-swimming beluga whales (*Delphinapterus leucas*)

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Presentation type: Oral Presentation

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Abstract:

Climate change has caused significant warming in the Arctic, which has led to a decrease in sea-ice extent and duration, increase in predators and human activity, changes in prey distribution, and the emergence of infectious disease. These factors have the potential to cause stress in marine mammals, ultimately impacting the health and fitness of the population. Cortisol is often used as an indicator of health, as elevated levels can indicate environmental stressors. The objectives of this project are to (1) develop a method to collect blow from wild, free-swimming beluga whales; (2) measure cortisol from blow samples; and (3) examine cortisol levels in relation to sampling device, number of exhalations, age class, time and date, and ship presence. Blow samples were collected from beluga whales in Western Hudson Bay by placing a collection device (petri dish or petri dish with nitex membrane) over the blowhole of belugas as they surfaced for air. Cortisol was extracted with ethanol and measured using enzyme immunoassays. We collected 252 blow samples from free-swimming beluga whales, making it the first time that it had been done. Cortisol concentrations ranged between the limit of detection (29.44 pg/ml) and 932.96 pg/ml. Samples collected on nitex had significantly higher cortisol concentrations than those collected on petri dishes ($P < 0.001$), after accounting for assay interference. Cortisol concentrations increased with the number of exhalations and differed between age groups. Based on these preliminary results, we conclude that blow collection is a feasible technique for measuring cortisol in free-swimming beluga whales; however, sampling techniques, such as the type of collection device used and the number of exhalations collected, can influence cortisol concentrations.

Non-invasive assessment of physiological stress in captive Asian elephants

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Abstract:

Asian elephant populations continue to decline in many parts of the world due to loss and fragmentation of habitats and poaching. Captive populations have also dropped drastically due to failure in reproduction, diseases, and mismanagement of reproductively active individuals however the demand for captive elephants is increasing. The present study aimed to examine the physiological stress response of captive elephants due to different husbandry practices, age/sex, captive/wild born, stereotypic behaviour and body conditions from four elephant camps/facilities. Approximately 870 dung samples of 37 captive elephants (24 males and 13 females) from four facilities were collected to examine fecal glucocorticoid metabolites concentration (fGCM). Fecal glucocorticoid metabolites were extracted using previously reported method and fecal hormone metabolites were analysed by EIA using Cortisol-R4866 antibody (Munro, UC Davis, USA) as previously reported procedure. The general linear model (GLM) procedure with repeated measures was used to examine relationship with hormone concentrations within and between the facilities with reference to age/ sex, working conditions and body condition score. Overall, fGCM concentrations significantly varied with different physical activities ($F_{2,434}=4.26$ $P=0.039$), between facilities ($F_{5,896}=4.2$, $P=0.03$) and body conditions ($F_{5,896}=6.6$, $P=0.001$). Further, the elephants born in the wild had significantly higher fGCM than captive-born in all facilities (M-W U test $P<0.05$ for all). The Dussehra elephants (participates in regular pubic processions) had significantly higher mean fGCM concentration (36.26 ± 1.7 ng/g) than the Mysore zoo elephants (14.7 ± 1.3 , M-W U test $P=0.001$). Further, fGCM significantly influenced by working hours ($F_{1,81}=5.7$, $P=0.019$), weight carried during the procession ($F_{3,81}=3.4$, $P=0.021$) and Body Score conditions ($F_{5,81}=2.7$, $P=0.024$) but not with age ($F=1.23$; $P=0.268$, sex (GLM $F_{1,81}=0.05$, $P=0.943$) or stereotypic behaviour of animals ($F_{1,81}=0.644$, $P=0.421$). The management recommendation includes a complete ban on participation in religious activities, public processions, and film shooting activities.

Hairs do not contain a historical record of stress – elucidating hair glucocorticoid kinetics in laboratory rats

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Presentation type: Oral Presentation

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Abstract:

Measuring glucocorticoids (GCs) in hairs has, over time, become the most popular method for non-invasive biomarker-based stress assessments in vertebrates. It is frequently assumed that hair glucocorticoids (hGCs) integrate the HPA-axis activity throughout the entirety of a hair's growth, with GC concentrations in cross-sections of hair reflecting specific periods in the past. Yet, stringent biological validation of the method and these assumptions are all but completely missing. In a laboratory setting, we investigated the kinetics of GC uptake into hairs, attempting to create an evidence-based model. Male rats with no adrenals, and consequently no endogenous production of GCs, were dosed, systemically, with high concentrations of corticosterone. Three hours following the first treatment, GC concentrations in hairs sampled from the lower back were significantly increased. hGC concentrations increased until daily treatments were stopped on day seven. The built-up GC levels were subsequently eliminated from the rats' hairs. One week after stopping treatments, hGC concentrations had fallen significantly. Past this point, hGC levels were indistinguishable from the response seen within 24 hours of the first treatment. The exact kinetics of hGCs may be subtly different for different species, but we can unequivocally conclude that hGCs are not a historical marker of stress. GCs diffuse freely in and out of hairs. There is no "stress calendar" in hairs and hGC concentrations from major stressors that happened days in the past are easily over-shadowed by those resulting from transient, acute stressors. The method is certainly a useful one with many convenient aspects – easy sampling, easy storage of samples, ability to quantitate stressors several days in the past, etc. – but the method assesses ongoing stress, not historical events. Going forward, we recommend taking the updated model of hGC kinetics into account and practicing care when interpreting GC concentrations in hairs.

Non-invasive monitoring of physiological stress in an Afrotropical arid-zone passerine bird, the southern pied babbler (*Turdoides bicolor*)

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Presentation type: Oral Presentation

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Abstract:

Using faecal matter to monitor stress levels in birds is a powerful technique that has been used for elucidating the effects of biotic and abiotic stressors over the last three decades. The aim of this study was to evaluate the suitability of five enzyme immunoassays (EIAs) for measuring faecal glucocorticoid metabolite (fGCM) concentrations to monitor adrenocortical activity in southern pied babblers. We performed a physiological validation on captive babblers through an ACTH challenge, and a biological validation using individually housed babblers temporarily separated from their social group. Additionally, we compared fGCM concentrations of both captive and wild babblers and of dominant and subordinate wild individuals to determine the effects of captivity and dominance status respectively. We identified two EIAs (detecting antibodies against 11-oxo-aetiocholanolone-17-CMO:BSA and 5b-pregnane-3a,11b,21-triol-20-one-CMO:BSA) to perform adequately and showed through the ACTH challenge that droppings were a suitable matrix for measuring physiological stress ($Z = -1.58$, $P < 0.05$). We found the effect of temporary separation from the group for this highly social species caused a 3-4-fold increase in fGCM levels ($Z = -3.52$, $P < 0.001$). When comparing wild ($n = 46$) to captive ($n = 10$) babblers, we found that individuals temporarily held in captivity had 30- to 50-fold higher fGCM concentrations than wild individuals ($Z = -2.10$, $P < 0.001$), indicating that captive babblers experience high levels of stress. When investigating the effect of dominant status, we found that in wild, free-living individuals, dominant males ($n=8$) showed the highest levels of stress ($t = 2.276$, $P < 0.05$), with no significant difference among the other groups, suggesting that being the dominant male of a highly territorial social group is stressful. Non-invasive sampling allows field-based researchers to reduce disturbance related to monitoring adrenocortical function, however in social birds, constraint and capture stress has to be carefully considered.

Validation of an enzyme immunoassay (EIA) for hair glucocorticoids determination in a wild population of Egyptian mongoose

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Presentation type: Oral Presentation

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Abstract:

Hair glucocorticoids (hGC) are increasingly used to understand the effect of natural and anthropogenic stressors on wild animals, but it is potentially confounded by individual, seasonal and sex-dependant variations. Therefore, we aimed to validate an EIA for hGC in a medium-sized carnivore, the Egyptian mongoose (*Herpestes ichneumon*). Hair samples (n=294) were collected in Portugal from specimens that had been immediately frozen after death. After thawing, hair was clipped between the shoulders and stored until extraction. Full-length guard hairs were washed twice with 90% methanol. Ten mg of hair was milled to powder and extracted with 90% methanol. Supernatants were diluted to 45% methanol. For LC-MS/MS analysis, randomly chosen samples from males (n=7) and females (n=7) were dried and resolved in 500µL methanol. HPLC-immunograms were performed on a pooled sample (corresponding to 550 mg hair). Two in-house immunoassays based on antibodies against cortisol-3-CMO- and cortisol-21-HS-BSA were used to determine immunoreactivities in HPLC-fractions. LC-MS/MS analysis revealed that cortisol and corticosterone were below detection level in half of the chosen samples. Cortisone (2.9-18.3 pg/g) and DHEA (171.6–278.8 pg/g) were measurable in all samples. Cortisone values were significantly correlated to concentrations obtained with the cortisol-3-CMO-EIA ($R^2=0.79$; $P<0.001$), whereas DHEA did not show any relationship ($R^2=0.033$; $P>0.05$). Using the 21-HS-assay, several unknown peaks of immunoreactivity were detected, with cortisol (6.8%) and cortisone (2.8%) representing only minor peaks. With the 3-CMO-assay, cortisol was the most prominent peak (38%), followed by cortisone (9.0%). All mongoose samples were analysed using both cortisol-EIA. Statistical analyses revealed that age, sex and storage time, but not season, had an effect on hGC if determined by 3-CMO-EIA. In contrast, the 21-HS-cortisol-EIA revealed only the effect of age. By identifying the most suitable EIA for hGC, we are now able to apply hGC to understand the effect of stressors on the wild population.

Adiposity, reproductive cycling status, and activity levels in zoo Asian elephants (*Elephas maximus*)

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Presentation type: Oral Presentation

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Abstract:

Asian elephant captive populations are not self-sustaining, possibly due to obesity related health and reproductive issues. The objective of this study was to estimate body composition and investigate the relationship between fat mass with glucose, insulin, inflammation, and activity levels in Asian elephants. Additionally, to examine the association between cycling status and fat in females. In 37 (n=28 females; n=9 males) elephants, deuterium dilution was used to estimate body composition based on total body water (TBW). TBW was divided by the mammalian hydration coefficient (0.73) to calculate fat free mass (FFM). FFM was then subtracted from weight to infer fat. Each elephant was weighed, ingested a single dose of deuterated water orally (0.05 mL/kg), and blood was collected prior to and five times after deuterium administration. The same vein location (i.e., ear or leg) was used consistently within an elephant for the sequential blood collections. Serum glucose, insulin, serum amyloid A, and activity levels were assessed. Activity levels were measured by accelerometer placed on the elephant's front leg for two days. Body fat percentage ranged from 3.54% to 24.59%. Males were significantly heavier ($P=0.032$), with significantly more FFM ($P=0.011$), but not fat ($P=0.919$) compared to females. For all elephants, absolute fat ($\rho=0.381$, $P=0.031$) and relative fat ($\rho=0.463$, $P=0.008$) were correlated with serum insulin. Distance walked was negatively correlated with age ($\rho=-0.351$, $P=0.029$) and nearly reached significance with fat ($\rho=-0.349$, $P=0.069$) for all elephants. Fat adjusted for FFM ($P=0.016$), in addition to adjusted for FFM and age ($P<0.001$), were significantly associated with cycling status in females. Deuterium dilution appears to be a tenable body composition technique for Asian elephants. In this sample, fat predicted cycling status, with non-cycling elephants tending to have less fat compared to cycling elephants, and greater activity levels may contribute to lower fat.

Acute phase proteins as indicators of the elephant immune response to infectious disease

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Presentation type: Oral Presentation

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Abstract:

Elephants are susceptible to infectious diseases including elephant endotheliotropic herpesvirus (EEHV) and tuberculosis (TB). EEHV is the leading cause of death for juvenile Asian elephants, both under human care and in nature. TB is a zoonotic disease that poses a risk of transmission between elephants, humans and other species. Both diseases are difficult to manage, in part due to challenges with detecting infection, and to the complexity of underlying immune responses. Measuring biomarkers of immune function can be a useful approach to improve our understanding of disease processes in diverse species, and can be beneficial to disease management. Acute phase proteins form part of the innate immune response; they can be fast acting and increase by several orders of magnitude within hours of immune activation. For this study we measured two APPs, serum amyloid A and haptoglobin, in serum collected longitudinally from elephants with EEHV viremia (n=14) and TB (n=4) as biomarkers of the innate immune response. Overall, APPs were positively correlated with EEHV viremia, and differences in magnitude of response between individuals may be associated with the viral strain (EEHV1 vs. EEHV5) and severity of infection. Serum amyloid A often increased in serum prior to detectable viremia (as determined via qPCR), and so could be a useful early indicator of infection. The APP response during TB infection was less clear due to additional pathologies that occurred during the study period, and the subclinical nature of infection. However, increases in APPs also occurred during the time that latent infection was suspected to convert to active disease, and with disease progression. Earlier detection of these pathologies could improve survival (EEHV) and reduce risk of transmission (TB). APPs are sensitive indicators of immune function in elephants that could be powerful tools to improve our understanding and management of infectious disease.

Predicting all-cause disease, CVD, and mortality risk in zoo-housed western lowland gorillas (*Gorilla gorilla gorilla*) using lipid and inflammatory markers

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Presentation type: Oral Presentation

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Abstract:

In humans, lipid and inflammatory markers predict risk of chronic disease (e.g., arthritis), including CVD, and mortality. Such markers may also provide valuable health information in great apes given their similar morbidities. Multiple lipid (cholesterol, triglycerides, high-density lipoprotein [HDL], low-density lipoprotein [LDL], apolipoprotein A1 [APOA1], total cholesterol/HDL ratio) and inflammatory (albumin, C-reactive protein [CRP], interleukin-6 [IL-6], tumor necrosis factor- α [TNF- α]) markers were measured from gorilla serum samples collected during routine exams (n=48, males=23, 1 sample/gorilla, aged 6-52 years). We examined associations with age and sex using linear regression or GLMs ($\alpha=0.05$). Age significantly negatively associated with albumin, HDL, and APOA1, and significantly positively associated with IL-6, triglycerides, and cholesterol/HDL ratio. In males, HDL and albumin were significantly higher, while CRP, TNF- α , and cholesterol/HDL ratio were significantly lower. Using multi-model inference, we ranked GLMs with binomial distributions and logit links to determine if lipid and inflammatory markers predict all-cause disease, CVD, and mortality risk. The first model contained sex and age, subsequent models added individual markers, and a global model contained all markers (lipid and inflammatory markers were analyzed separately). With inflammatory markers, the top model for all-cause disease and mortality risk contained sex and age only, while CVD risk was best predicted by models containing age, sex, and TNF- α . All except the global models were within the top model set. For lipid markers, all models except the global models were within the top model set for each health outcome. As models within the top model set ($\Delta AICc \leq 6$), especially those $\Delta AICc \leq 2$, may explain risk as well as the top model, these results suggest that although lipid and inflammatory markers predict all-cause disease and mortality risk in gorillas, they may not be more informative than age and sex alone. However, CVD risk may be best predicted by models containing age, sex, and TNF- α .

Baleen hormone cycles inform reproductive histories of female bowhead whales

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Presentation type: Oral Presentation

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Abstract:

Reproductive life histories of large, long-lived marine mammals such as baleen whales are difficult to determine. Recent validation studies have shown that longitudinal profiles of reproductive and stress hormones in baleen match past calving history and exposure to natural and anthropogenic stressors such as pregnancy and gear entanglement. We measured long-term hormone profiles in 2-cm increments along baleen plates of 10 female bowhead whales (*Balaena mysticetus*) from the Eastern Canada-West Greenland (EC-WG) population. Our primary objective was to assess calving rates from progesterone concentrations, but estradiol and corticosterone were also measured to provide additional details on reproductive patterns (e.g. estrous). Pronounced progesterone peaks occurred in seven of the 10 females, with an average amplitude of approximately 1000 ng/g and spacing ranging from 46 to 66 cm. Assuming a constant annual growth rate of approximately 18 cm/yr (determined previously for these plates using annual stable isotope cycles), progesterone peaks corresponded to a calving interval of approximately 2.6 to 3.7 years. Interestingly, the three females with uniformly low baleen progesterone concentrations came from an area where immature animals aggregate during the sampling period, supporting previous findings of seasonal sex and age-class segregation in this population. Concurrent peaks in corticosterone and estradiol occurred within the latter half of most progesterone peaks, when they may reflect stress or maintenance of pregnancy. Shorter progesterone peaks that did not have an accompanying estradiol peak were followed closely by another progesterone peak that did, suggesting a failed pregnancy that was followed by a successful one. EC-WG bowheads are considered depleted from commercial whaling and are subject to a small subsistence hunt by Canadian Inuit. These hormone-derived estimates, which are the first population-specific estimates of reproductive rates for EC-WG bowheads, will inform population growth models used to manage their recovery while maintaining culturally important hunts.

Immunocontraception of male and female giraffes using the GnRH vaccine Improvac®

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Presentation type: Oral Presentation

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Abstract:

Vaccination against GnRH (Gonadotropin releasing hormone) inhibits the production of the gonadotropins LH and FSH, and thus gonadal steroids. These type of immunizations have been extensively studied in horses, boars, dogs, cats and to a lesser extent in wildlife i.e. white-tailed deer, African elephants, and the Indian rhinoceros. This contraceptive study evaluated the effects of the GnRH vaccine Improvac® on testicular and ovarian activity in male (n = 9) and female (n = 18) giraffes (*Giraffa camelopardalis*) kept in European zoos. The contraceptive efficacy was tested by measuring faecal testosterone and progesterone metabolites. At the beginning of this study vaccination intervals were based on protocols used in horses (2-3 shots for basic immunization, followed by booster injections after 4 to 6 months). For effective contraception in giraffes, these intervals appeared to be too long. A reliable reduction of faecal steroid metabolites to baseline levels occurred only after 3 – 4 Improvac® injections. Recommendations for the immunization protocols were adapted and good immunization responses were achieved, when basic immunization of 3 injections within 8 weeks were followed by booster injections in 8 – 10 week intervals. Long-term (>2years) use in females was often accompanied by prolonged periods of persistent corpus luteum activity, although normal cycles were not observed. Problems might occur with reversibility, because in some male and females, even after more than 2 years since treatment was stopped, androgen and progesterone metabolite levels have not returned to normal values. The result is somewhat ambiguous, on the one hand the reproduction can be suppressed, but the question of reversibility is unclear.

Unraveling the giant panda reproductive biology: potential roles of prostaglandins PGE₂ and PGF_{2α} – preliminary results

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Presentation type: Oral Presentation

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Abstract:

In absence of a luteolytic factor, maternal recognition is missing in dogs with continued high progesterone production by the corpus luteum (CL) during (pseudo)pregnancy. We suggest a similar reproductive physiology in the phylogenetically related giant panda (GP). However, GPs additionally show a low-progesterone CL dormancy phase preceding the active luteal phase. The signals involved in regulating this biphasic progesterone secretion are not known. Nevertheless, we hypothesize an endocrinologically pre-programmed profile until the first 13,14-dihydro-15-keto-PGF_{2α} (PGFM) peak. In this study, we focused on the eicosanoid profile during the active luteal phase investigating the potential role of prostaglandin E₂ (PGE₂) as luteotropin in CL reactivation, likely contributing to the early PGFM peak (cross-reactivity), and in attachment/embryonal development when successfully bred, and of PGFM as luteolytic factor towards end-of-cycle. Immunoassays were used for endocrine profiling (estrogen, progesterone and PGFM). Four GPs (12 cycles) were included pursuing full-cycle daily urine samples. A PGE₂-immunoassay was performed on 3 GPs' active luteal phase samples (5 cycles; n=85), with parallel screening for prostaglandins and metabolites using ultra-high performance liquid chromatography - high resolution mass spectrometry (UHPLC-HRMS). PGE₂ significantly increased from baseline prior to changes in PGFM, indeed suggesting a driving role of PGE₂ during this phase. After the PGFM peak, in birth-cycles (n=2) an average 3.63-fold increase, compared to 1.32 in non-births (n=3), was observed. The PGE₂ concentrations were low (pg_{PGE2} versus ng/mL_{PGFM}) because of the known rapid metabolism of eicosanoids. Screening with UHPLC-HRMS confirmed the presence of PGE₂-metabolites. Further identification/quantification is pursued. PGFM showed a

significantly different profile in pregnancy with consistently increasing concentrations. This can be explained by a growing fetal stress response towards birth, whereby increased fetal cortisol initiates the progesterone block removal and myometrial activity by placental progesterone conversion to estrogens and production of $\text{PGF}_{2\alpha}$. In GP, a discriminative profile is thus not to be expected sooner than fetal/placental involvement in late pregnancy.

Study of reproductive endocrinology in sloth bear females through urinary estradiol and progesterone profiling

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Presentation type: Oral Presentation

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Abstract:

Sloth bears are one of the four bear species found in India. They are categorized as Vulnerable in the IUCN red list. Very little is known about sloth bear biology, including their basic reproductive endocrinology. In 2015 a research project was initiated at Agra Bear Rescue Facility in Uttar Pradesh, India to study female sloth bear urinary hormone profiles. Samples were collected non-invasively every day during the breeding season (April – July) and twice a week during the non-breeding season. Urinary estradiol and progesterone were quantified with EIA kits and the hormone levels were indexed against creatinine to account for variation in sample concentration. Estradiol profiles of 10, 13 and 6 bears were generated in 2016, 2017 and 2018, respectively. Progesterone of 6 and 5 bears was analysed in 2016 and 2017, respectively. All but one females were housed in mixed sex groups during the study period. However, all males were neutered, so no females mated or gave birth. Estradiol profiles indicated that female sloth bears experience a single annual estrus between May and July with most females reaching peak urinary estradiol levels in June. However, one female achieved this peak in August, indicating that the breeding season may be substantially prolonged. Progesterone profiling showed an increase in urinary progesterone levels for periods ranging from 85 - 191 days, mostly during the months of August to December. These sloth bear hormone profiles mimic the extended period between estrus and prolonged progesterone elevation recorded for the giant panda, and indicate that sloth bears also experience pseudopregnancy. The delay in the rise of progesterone likely reflects a period of corpus luteum inactivity, which is characteristic of delayed implantation in the giant panda and the American black bear. To our knowledge, this is the first report of reproductive endocrinology in sloth bear females.

Role of male androgen production in female mate choice in the endangered black-footed ferret

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Presentation type: Oral Presentation

Presenting author: Emily Potratz

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Abstract:

Black-footed ferrets (*Mustela nigripes*; BFF) are an endangered carnivore dependent on *ex situ* breeding for reestablishing wild, self-sustaining populations across North America. However, BFF recovery has been limited by a reduction in *ex situ* breeding success, with pregnancy rates decreasing from 70% to 35% over the last 20 years. We predict a lack of female mate choice is contributing, as pairings are based solely on pedigree. We aimed to determine how reinstating mate choice in *ex situ* breeding influenced reproductive success. Our objective was to determine if male testosterone concentrations (via fecal androgen metabolites, FAM) influenced female preference, successful breeding and litter production. We hypothesize that because androgens play a role in body condition, courtship behavior, and other sexual traits, then females would select males with higher FAMs to ensure litter production. BFFs (12 males: 12 females) were offered soiled bedding (feces, urine, gland secretions) from two opposite sex conspecifics using a “Y” maze. Preference was determined by time enacting exploratory or sexual behaviors such as sniffing, burrowing, and marking. Fecal samples were collected during the week of trials and analyzed for FAMs (Munro R167/8). Results showed that interaction with male scent increased female whelping success (58%) compared to pedigree-based pairings (40%); but didn't affect litter size (3.5 to 3.8 kits/litter, mate choice vs. pedigree-based pairs, respectively). While not significant, results from student t-test showed successful females preferred males with higher FAMs (16.58 ± 1.74 ug/g) than males with lower FAMs (12.35 ± 1.98 ug/g) ($t = -1.49$, $P = 0.14$). These results suggest hormone production may be discerned by BFFs, an olfactory dominant species, and used to determine quality and compatibility. This first step in exploring what factors affect female mate choice can be used to improve reproductive success, which would then provide more individuals for reintroduction into the wild.

Hormones in blubber tissue: a new tool for physiological assessment of the endangered North Atlantic right whale (*Eubalaena glacialis*)

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Presentation type: Oral Presentation

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Abstract:

Given the continued population decline of North Atlantic right whales (*Eubalaena glacialis*), developing a suite of tools to monitor physiology and health of the population is critical. Blubber offers an alternative sample type and may represent a unique timescale of endocrine data not captured by other validated matrices (feces, respiratory vapor, baleen) for Egs. We performed assay validations to measure testosterone, progesterone, and cortisol in Eg blubber using Arbor Assays ISWE mini-kits (#ISWE001, ISWE003, ISWE002). To help refine the study of whale blubber, we compared two extraction protocols on homogenized blubber tissue (0.07-0.12 g), involving (A): an extended protocol widely used in cetacean blubber studies, versus (B): a simplified extraction. Finally, we conducted a preliminary analysis of archived samples from known Egs (n=26). Results showed serially-diluted samples and standard curves were parallel and similar across extractions (A vs B), testosterone: P=0.68 vs P=0.30; progesterone: P=0.56 vs P=0.44; cortisol: P=0.76 vs P=0.79. Intra-sample variation was 1.8–3 times lower using extraction B compared to A. Accuracy test results for extraction B had slopes of 0.84, 0.73, and 0.91 (testosterone, progesterone, cortisol). Mean (\pm SE) testosterone concentrations in blubber from adult males (2.54 ± 0.50 ng/g) were approximately 2 times greater than juvenile males and 3 times greater than females. The highest progesterone value (60.30 ng/g) was measured in a confirmed pregnant female and was 13 times higher than mean progesterone of all other females (4.37 ± 0.95 ng/g). Blubber collected during necropsies of three whales with evidence of entanglement averaged 5.94 ± 1.50 ng/g cortisol compared to 0.72 ± 0.18 ng/g in live whales. Ultimately, extraction B may provide a more efficient and repeatable method for blubber hormone measurement. Further, data suggest differences in reproductive and stress-related hormones are detectable in Eg blubber, providing new application for monitoring changes in reproductive rates or responses to anthropogenic and environmental threats.

Preliminary analyses of serum phytoestrogens in white rhinoceros using liquid chromatography-multiple reaction monitoring mass spectrometry

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Presentation type: Oral Presentation

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Abstract:

Plants contain estrogen-like molecules that can bind to mammalian estrogen receptors and impact reproductive health. Exposure of human-managed herbivores to dietary phytoestrogens is inevitable as they consume large amounts of forage and, often, soy-based grain concentrates. Phytoestrogens are proposed to be a factor in decreased fertility of southern white rhinoceros (SWR; *Ceratotherium simum simum*) in zoos, however no data exists on the type or amount of circulating phytoestrogens in SWR. This study's objective was to quantify serum phytoestrogens in mature, non-pregnant female SWR at two North American institutions, White Oak (WO; n=9) and North Carolina Zoo (NC; n=4). Diets consisted of a grain concentrate (4 kg, WO; 0.45 kg, NC), pasture grass, and non-legume hay (35-45 kg, WO; 25-45 kg, NC). Serum samples were analyzed for phytoestrogen composition using targeted high performance liquid chromatography-multiple reaction monitoring mass spectrometry (LC-MRM). Genistein ranged from 0–121 ng/mL (mean \pm SE, 52.4 ± 14 ng/mL) for SWR at WO, and 0–174 ng/mL (64 ± 32 ng/mL) for SWR at NC. Equol, a potent metabolite of daidzein, ranged from 53–104 ng/mL (73.9 ± 7 ng/mL) for SWR at WO, and 48–253 ng/mL (134.1 ± 48 ng/mL) for SWR at NC. Results confirm that dietary phytoestrogens are reflected in SWR serum and vary widely among individuals, suggesting a complex relationship between phytoestrogen consumption and metabolism that requires further study. Although SWR at NC consumed less grain, their phytoestrogen concentrations appeared higher than those measured in SWR at WO, suggesting the type of forage and specific ingredients in grain concentrates influence serum phytoestrogen concentrations. This analysis is the first to apply LC-MRM technology to rhinoceros serum and report on circulating genistein and equol concentrations, an important step toward understanding the potential biological effects of circulating phytoestrogen concentrations in SWR.

Hair cortisol analyses in different mammal species: choosing the wrong enzyme immunoassay (EIA) may lead to erroneous results.

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Presentation type: Oral Presentation

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Abstract:

One way to study the effects of environmental stressors on wild animals is the measurement of cortisol in hair (hGC) which provides an integrated value of circulating cortisol over a period of several weeks and thus is unaffected by acute stressors. The most commonly employed methods are EIAs. However, given the cross-reactivity of similar steroids with antibodies used in EIAs, their relatively low specificity may result in misestimations. We compared two in-house immunoassays based on antibodies directed against cortisol-3-CMO- and cortisol-21-HS-BSA for hGC measurements in hairs of fattening rabbits and obtained 11-fold higher concentrations when applying the 21-HS assay (30.2 vs 2.7 pg/mg, respectively). Therefore, we ran HPLC immunograms to characterize the compounds detected by the respective EIA. In the rabbit, HPLC immunograms revealed huge portions of unpolar immunoreactivities when using the 21-HS-assay, with cortisol representing only a minor proportion of about 15% of total immunoreactivity. Clearly different results were obtained with the 3-CMO-assay, identifying cortisol (26%) and cortisone (12%) as the most prominent peaks. Similar results were revealed from hairs of other mammal species. To confirm the 3-CMO-assay as the most appropriate, we introduced high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) as golden standard for specific analyses of hGC in hair samples within the framework of a multi-species approach (Egyptian mongoose, Iberian lynx, cheetah, black bear, spotted hyena, Alpine marmot). In all species and samples (n=71) we obtained highly significant correlations between the cortisol-3-CMO EIA and LC-MS/MS-cortisone (R square >0.8) and LC-MS/MS-cortisol (R square >0.6), respectively. Cortisone was detected in all samples of all species but in quantitatively higher amounts compared to cortisol. Thus, our 3-CMO EIA is an appropriate assay to detect cortisol and cortisone in hairs of mammal species. However, the use of a cortisone-specific antibody might further improve stress objectification in hairs of mammals.

Who's poo? Using progesterone metabolites measured in faecal samples of paired captive short-beaked echidnas as a marker of sex

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Abstract:

The objective of this study was to determine whether differences in faecal hormone metabolite levels could be used to identify samples from male and female echidnas when these animals were paired for the breeding season. A pilot study evaluating testosterone and oestradiol metabolites in echidna faeces found considerable overlap between the two sexes and were considered unsuitable. A previous study has revealed that female echidna faecal samples contain higher progesterone concentrations than that of males; therefore, progesterone was investigated as an alternative hormone to identify sex. Faecal samples were collected at various intervals between August 2017 and January 2019 from nine male and 10 female echidnas. These samples were analysed by enzyme-immunoassay using two antibodies (R4859 and CL425; CJ Munro, USA) to determine the most suitable for this species. Using the progesterone specific antibody (R4859), there was no significant difference in mean (\pm SEM) progesterone metabolite values between males and females during the breeding ($1,479 \pm 294$ ng/g vs $1,054 \pm 261$ ng/g, $p = 0.2960$) and non-breeding seasons (539 ± 335 ng/g vs 864 ± 195 ng/g, $p = 0.3950$), respectively. In contrast, use of the broad-spectrum progesterone antibody (CL425) produced progesterone metabolite levels that were significantly higher in males than females during the breeding ($6,141 \pm 667$ ng/g vs 755 ± 184 ng/g, $P < 0.001$) and non-breeding seasons ($2,684 \pm 616$ ng/g vs 725 ± 160 ng/g, $P < 0.001$), respectively. While somewhat counter-intuitive, higher progesterone metabolite excretion in males may serve to be a reliable method of sex identification in captive echidnas and could have an important biological role in modulating male sexual behaviour. Further analysis of breeding season samples coupled with faecal DNA confirmation of ID is underway, as are studies to determine the relevance and source of elevated progesterone concentration in male echidnas.

Measurement and preliminary validation of 1α -hydroxycorticosterone (1α OH-B) stress hormone in elasmobranchs

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Abstract:

Measurement of 1α -hydroxycorticosterone (1α OH-B) has been of significant biological interest and a major scientific challenge for over 50 years. Currently, elasmobranch stress evaluations have been limited to behavior and secondary stressors, primarily blood gasses. Although commercial enzyme immunoassays (EIA) are available for corticosterone, those tested exhibit low relative cross-reactivity to 1α OH-B (<5%). To improve measurement, a monoclonal antibody and in-house EIA was developed and used to validate sample extraction protocols to optimize measurement of 1α OH-B in <1ml blood sample. Banked serum and plasma samples from aquarium-managed bonnethead and scalloped hammerhead sharks, and southern stingrays (The SEAS with Nemo and Friends[®]; *Sphyrna tiburo*, N=10; *Sphyrna lewini*, N=3; *Hypanus americanus*, N=4), or semi-wild (habituated; Disney's Castaway Cay, Commonwealth of the Bahamas**, N=18), and wild *H. americanus* (Bimini, N=18) populations were assessed for 1α OH-B to test expected variability between species, populations, health conditions (eg. illness, injury), and correlations with secondary stress blood values. Initial findings show that 1α OH-B: 1) has species-specific concentrations and ranges; 2) exhibits a reduced range in habituated populations; 3) is elevated during illness or injury; and 4) increases in a linear fashion with species-specific increases in blood glucose and lactate values, and decreasing pH values. Together, these data support a stress hormone role for 1α OH-B in elasmobranchs. Measurement of 1α OH-B may be a valuable tool to evaluate stress hormone changes and health and welfare in elasmobranchs which will be important for many aspects of collection, transport, medical treatment, aquaria and conservation management of these charismatic and ecologically important species.

Findings from long term reproductive and adrenal hormone monitoring of Asian elephants at the National Zoological Park and Oregon Zoo

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Presentation type: Oral Presentation

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Abstract:

Through longitudinal hormone monitoring, much is known about basic endocrine function in elephants, information that has been used to improve captive management through better timing of breeding (both natural and assisted), increased social opportunities, and improvements in exhibit design and husbandry. Using banked serum at the Oregon Zoo and National Zoo, we summarized over 20 years of progestagen and cortisol data in 13 female, and testosterone and cortisol data in four male Asian elephants from puberty through senescence with an age span of 4 to 71 years to assess reproductive and adrenal responses to environmental and physiological factors. All adult bulls historically displayed annual musth, and the majority of females cycled normally. Changes in serum testosterone were associated with development and aging. The youngest male showed increases in testosterone during early musth cycles, starting at 15 years of age; the oldest male, age 32 to 54 years, exhibited a gradual decline in testosterone concentrations during musth with more erratic visible and behavioral signs. Tuberculosis treatment in three bulls had no apparent effect on testosterone production. In females, estrous cycle characteristics were more varied than previously reported, and the follicular phase appeared to have a regulatory role in maintaining a consistent cycle duration. The first pubertal cycle was similar to subsequent cycles, and cycle durations did not appear to change with age. Through GLMM analysis we found that reproductive status (prepubertal, cycling, pregnant, lactational anestrous, acyclic) and cycle phase were predictors of cortisol concentration, with higher concentrations during the follicular phase. Major life events had minimal effects on female cycle dynamics or cortisol concentrations. We are now assessing variation in serum cortisol in relation to musth cycles and age, and adrenal responses to major life events, social changes, and medical treatment, which may have implications for how we manage male elephants.

Exploring relationships between fecal glucocorticoid metabolites, social coping mechanisms, and a novel, non-invasive measure of oxidative stress in grizzly bears (*Ursus arctos horribilis*)

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Presentation type: Oral Presentation

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Abstract:

Measures of oxidative stress have potential for integrating positive and negative life experiences into cumulative indicators of animal welfare. Glucocorticoids may contribute to oxidative stress—causally linking environmental stress to physiological aging via molecular damage—but this relationship needs further study. We explored these concepts when three, male, eight year-old grizzly bear littermates at the Detroit Zoo were moved to a smaller habitat for three months while their previous home was expanded. We hypothesized that spatial compression and construction activity during this time would cause stress. We predicted that the bears might use social behavior as a coping mechanism, so rates of play would correlate with physiological measures of stress. Every weekday, we conducted two hours of behavioral observations using scan and all-occurrences sampling and collected fecal samples. We analyzed a subset of data when both behavior and fecal samples were available: fourteen days per bear. We tested fecal glucocorticoid metabolites (FGM) using a cortisol enzyme-immunoassay (ISWE002, Arbor Assays) validated via parallelism (ANCOVA, $F(1,11)=0.01$, $P=0.94$) and accuracy/recovery (averaging 108%). We tested for oxidative stress in fecal extracts using an enzyme-immunoassay (K059, Arbor Assays) targeting a byproduct of DNA damage, 8-hydroxy-2'-deoxyguanosine (8-OHdG), validated using parallelism (ANCOVA, $F(1,14)=607.12$, $P=0.423$) and accuracy/recovery (averaging 99%). A linear mixed model showed that log-transformed (log)8-OHdG concentrations increased significantly with higher log(FGM) concentrations ($F(1,36)=6.55$, $P=0.015$), supporting a relationship between adrenal activity and rates of DNA damage. Higher rates of social play exhibited a trend towards decreased log(8-OHdG) ($F(1,37)=3.23$, $P=0.080$) but not log(FGM), which is consistent with social behavior mitigating the molecular damage from stress. Individual dominance and the directionality of solicitations to play also may have influenced the results. Although these preliminary results require further validation, 8-OHdG shows promise as a non-invasive, cumulative indicator of animal welfare.

Validating the use of qiviut cortisol as a stress biomarker in muskoxen (*Ovibos moschatus*)

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Presentation type: Oral Presentation

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Abstract:

Muskoxen are increasingly exposed to stressors in the rapidly changing Arctic. The goal of this study was to establish the patterns of uptake/deposition, and stability of cortisol in qiviut (annually shed undercoat), so that it could serve as a tool for monitoring health. Our specific objectives were to (i) assess if cortisol deposition in qiviut differs between neck, shoulder and rump; (ii) determine if a repeated experimental increase of endogenous cortisol is reflected in qiviut; (iii) assess if cortisol is stable in qiviut over time; and (iv) determine if a single and repeated experimental increases of endogenous cortisol elevate qiviut cortisol levels when the hair is not growing. At the Large Animal Research Station we conducted two ACTH challenges on captive muskoxen, one in winter (Feb/Mar; no hair growth) and the other in summer (July/Aug; maximum hair growth). Animals were allocated to treatment (n=10) and control (n=5-winter or 6-summer) groups using a randomized block design. Treatment groups received weekly injections of slow-release ACTH during 5 consecutive weeks while controls received saline. During the winter challenge, hair samples were collected from the rump at first injection, 1 week, and 7 weeks. During the summer challenge, hair was collected from neck, shoulder, and rump at first injection and at 7 weeks. Rump hair was sampled again at 3 and 6 months post-challenge. Qiviut cortisol was analyzed by ELISA validated against mass spectrometry. Rump hair cortisol significantly increased from median 7.38 pg/mg to 14.64 pg/mg in treatment animals in summer (paired Wilcoxon signed-rank test: $P=0.002$) but not in controls (8.84 pg/mg versus 11.35 pg/mg post-challenge, $P=0.094$). By discussing these results, body-region and qiviut cortisol quantitation method differences, along with stability findings, we will improve our understanding of cortisol dynamics in hair. This will lay the ground for reliably interpreting results from wild animals.

Relationships of Nutrition to Fecal Glucocorticoid Metabolites in Bison (*Bison bison*)

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Presentation type: Oral Presentation

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Abstract:

Species experiencing dramatic changes in nutrient availability might experience concomitant changes in glucocorticoid secretion to metabolize nutrients for energy management. This study hypothesized that changes in female bison fecal glucocorticoid metabolite (FGM) concentrations were associated with nutrient availability across the year. FGM were measured monthly for one year in four bison herds (n=30 samples/herd) on natural rangeland, and nutritional quality of pasture samples was assessed. FGM also were measured in two yearling groups (n=30 samples/group) transitioned from pasture to either a high-energy corn or moderate-energy barley diet for seven months. Correlations between FGM and pasture quality were tested by Spearman correlation. Differences in FGM between months and groups were assessed by non-parametric ANOVA. FGM concentrations in each herd differed ($P < 0.001$) across months with seasonal pasture changes and were correlated with pasture nutritional quality (protein: $P \leq 0.03$, $R^2 \geq 0.7$, total digestible nutrients: $P \leq 0.03$, $R^2 \geq 0.7$, acid detergent fiber: $P \leq 0.04$, $R^2 < 0.6$). Experimental yearlings' FGM concentrations increased ($P < 0.001$) upon switching from pasture to feed. FGM concentrations were higher ($P < 0.05$) in barley compared to corn-fed yearlings and decreased more gradually summer to fall for barley compared to corn-fed yearlings. Interestingly, initial decreases ($P < 0.05$) in FGM in both yearling groups were consistent with when FGM began to decrease in rangeland bison, which might be coincidental, related to rumen acidosis, or a preserved evolutionary mechanism for seasonal energy conservation. This study demonstrates that FGM concentrations in female bison are directly correlated with changes in pasture nutritional quality. Yearlings on feed responded to increased nutrition with increased FGM, but the adrenal response of those on moderate-energy feed was higher than that of yearlings on high-energy feed, and the response of both groups diminished over time despite maintained nutrition. These data highlight the role of glucocorticoids in normal metabolic function and underscore the importance of considering nutrition when studying glucocorticoids.

Using ultrasonography and endocrinology to understand folliculogenesis and reproductive failure in whooping cranes

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Presentation type: Poster

Presenting author: Megan Brown

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Abstract:

For endangered species managed ex situ, production of offspring is a key factor to ensure healthy and self-sustaining populations. However, current breeding and reintroduction goals for the whooping crane (*Grus americana*) are impeded by poor reproduction. Our study seeks to better understand mechanisms regulating ovarian function in captive whooping cranes and the overall regulatory function of the HPG axis in relation to follicle formation and egg laying. We know from other avian species that small follicles on the ovary produce estradiol which in turn stimulates release of luteinizing hormone from the pituitary and yolk precursors (vitellogenin and very low-density lipoproteins) from the liver. As dominant follicles continue to grow, they enter the preovulatory phase and begin to incorporate yolk precursors, undergo cellular differentiation, and transition to progesterone production, while the remaining small follicles continue to produce estradiol. We hypothesize that non-laying females are experiencing a perturbed HPG axis which depresses steroid hormone production and prevents follicles from switching to this preovulatory phase. For this study, we paired weekly blood collections with a real time visualization of the ovary through transcutaneous ultrasonography in six adult female whooping cranes. Ultrasonic images were scored based on ovarian condition, number of follicles visible, and size of largest follicle. Plasma samples were analyzed using enzyme immunoassay with estradiol antibody R0008 and progesterone antibody CL425 (C. Monroe, UC Davis). Preovulatory follicles (>12mm) were observed in all laying females (n=3) but absent in non-laying females (n=3). Estradiol concentrations were significantly higher with advanced ovarian condition, while both estradiol and progesterone concentrations were elevated when the largest follicle reached 8mm in size ($p<0.001$) or when three or more supportive follicles were present on the ovary ($p<0.001$). Ongoing data analysis is investigating the patterns and roles of pituitary protein hormones and yolk precursors in follicle formation and egg production

**Are we expecting? Developing a test for pregnancy detection in captive snow leopards
(*Panthera uncia*)**

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Presentation type: Poster

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Abstract:

Reliable methods of pregnancy detection in captive snow leopards (*Panthera uncia*) are needed to inform animal management practices to improve reproductive success and cub survival. The reproductive cycles of snow leopards have been characterized; however, identification of pregnancy biomarkers and tests are still needed. Measurement of prostaglandin_{F_{2α}} metabolites (PGFM) has been used to determine pregnancy in other *Panthera* species, but success has been variable. We seek to validate the use of PGFM monitoring of urine and fecal samples to diagnosis pregnancy in snow leopards. Preliminary data for two pregnancies from one female demonstrated that urinary PGFM predictably increases approximately 55 days post-mating in pregnant versus pseudo-pregnant (i.e., ovulated without conception) snow leopards; and then continues to exponentially increase until birth. However, because collection of urine samples is often not feasible, we sought to develop a protocol for using fecal samples for monitoring reproductive cycles and diagnosing pregnancy. In total, we gathered urine samples from 2 females collected during 2 pregnancies and 3 pseudopregnancies; and fecal samples from 9 females at 8 institutions collected during 3 pregnancies, 2 still births, 3 pseudopregnancies, and 1 exogenously-hormone-induced ovulation. Using Arbor Assays PGFM kits and in-house progesterone assays we have characterized urine and fecal PGFM profiles of pregnant and pseudo-pregnant snow leopards. We found that like urinary PGFM, fecal PGFM is elevated during the last trimester of gestation and can be used to non-invasively diagnosis pregnancy in snow leopards. However, for the pregnancies characterized using fecal PGFM, the start of exponential increase in PGFM concentration indicative of pregnancy was variable, with the increase in fecal PGFM concentrations occurring as early as 56 days to as late as 71 days post-mating. Dual substrate comparisons during pregnancy are needed to determine if this variation is due to individual or the substrate used to measure PGFM.

Leave me alone - Association patterns influence reproductive and stress-related faecal hormone metabolite concentrations in giraffe bulls

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Presentation type: Poster

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Abstract:

Giraffes (*Giraffa camelopardalis*) live in a flexible fission-fusion system, characterized by the frequent splitting and reunion of population subgroups. Males assume a more solitary lifestyle and are often seen roaming alone between female groups. In this study, we applied non-invasive methods to monitor faecal androgen (fAM) and glucocorticoid (fGCM) metabolite levels of free-ranging giraffe bulls to examine longitudinal patterns of steroid metabolite concentrations in relation to observed male behaviour in different age classes. 585 faecal samples from 20 males were collected over 12 months and analysed using an Epiandrosterone (fAM) and 11-oxo-etiocholanolone II (fGCM) enzyme immunoassay. Additionally, association patterns between males and females on a population level were investigated using social network analyses. Males and females exhibited different association patterns, which varied with season, depending on female reproductive status. Different grouping patterns correlated with the endocrine milieu of the male giraffes. More all-male groups were present in winter and this is reflected in lower overall fAM concentrations for bulls in these groups compared to when in mixed sex groups (9.68 vs 5.39 $\mu\text{g/g DW}$, $P=0.003$). Androgen levels may increase due to the stimuli of fertile females present and in preparation of mating activity and mate guarding. fGCM levels in giraffe bulls are also associated with changes in the social environment. The youngest bulls exhibited the overall highest fGCM levels (young: 1.07 $\mu\text{g/g DW}$, old: 0.87 $\mu\text{g/g DW}$, $P=0.015$). However, when in mixed sex groups, the highest fGCM levels were found in the oldest bulls (young: 0.78 $\mu\text{g/g DW}$, old: 1.07 $\mu\text{g/g DW}$, $P=0.003$), which might be explainable to energy demanding behavior, such as mate guarding and that can lead to an increase in allostatic load. In summary, the highly flexible social environment influences the endocrine milieu of giraffe bulls, and these findings can be helpful for population management.

Is a GnRH Vaccine an effective contraception method in zoo-managed mammals?

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Presentation type: Poster

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Abstract:

Effective contraception plays a critical role in collection plans for zoo species. Permanent methods to control reproduction often are not suitable options, and short-term contraceptive efforts (e.g., synthetic progestins) have proved challenging. A promising alternative method for use in both sexes is a gonadotropin-releasing hormone (GnRH) immunocontraceptive vaccine. This vaccine has been used successfully for population control in a variety of wild and domestic mammalian species. We tested the efficacy of the GnRH vaccine GonaCon™: USDA in Nile hippopotamuses, (n=2♀, 1♂) or Improvest®: Zoetis US in red river hogs (n=2♀), large flying foxes (n=12♂), Nubian goat (n=1♀), warthogs (n=2♀), and reticulated giraffe (n=1♀). Animals were injected subcutaneously 1-3 times with the vaccine using empirical doses. Serial injections were separated by at least four weeks. Before and after vaccine administration, we measured fecal testosterone and progesterone in males and females, respectively, using enzyme immunoassays. Results were highly variable depending on species and/or course of treatment. For example, treatment was effective in all species tested with the exception of warthogs. Bats were suppressed but had negative vaccine reactions following a single injection of the vaccine. All other species required multiple doses to suppress patterns of gonadal steroids. GnRH vaccines have potential for contraceptive treatment in zoo mammals. Health and hormone monitoring, however, is needed in species previously untested to assess safety, efficacy, reversibility, and the need for booster injections.

Establishing a living Koala genome bank: non-invasive hormone monitoring as a management tool for breeding koalas in captivity

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Presentation type: Poster

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Abstract:

A “Living Koala Genome Bank” is a concept for capturing genetic variation and restoring population connectivity for the future preservation of local wild population genetics. The concept includes the strategic, genetic based, mating of wild and captive koalas. Wild koalas temporarily brought into captivity, are housed together with minimal human contact for mating purposes to produce offspring that will be released with their mother back into local habitat. Wild males are also paired with captive female koalas to infuse genetics from local wild populations into the captive population to create a genetic reservoir for future withdrawal. The aim of this project was to use non-invasive hormone monitoring as a management tool to evaluate breeding attempts in the research program. Faecal samples were collected 2-3 times per week from female koalas and stored frozen until processing. Samples were dried at 65°C overnight, weighed (0.2 g) and extracted with 5ml of 80% methanol overnight. The faecal extracts were analysed by enzyme-immunoassay using a broad spectrum progesterone antibody (CL425, Coralie Munro, UC Davis, USA). Significant increases of faecal progesterone metabolite levels were detected in females that were successfully induced to ovulate. A total of 11 luteal phases were detected after pairing for mating (n = 6 of 8 mating attempts) or during cohabitation (n = 4 of 5 females including n = 1 with two confirmed ovulations). From these, 4 females were confirmed with pouch young for a 36% success rate after ovulation. For wild koalas, detection of ovulation allowed for pouch checks to be strategically timed, to minimize handling and human contact. These techniques facilitated hands-off management of wild koalas housed temporarily in captivity for breeding, identified possible infertility (n = 1) and allowed for the assessment of ovulation rates versus pouch young production to evaluate breeding success parameters for this species.

Determining the effects of dietary phytoestrogens on red colobus monkey (*Piliocolobus tephrosceles*) fecal hormone levels in Kibale National Park, Uganda

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Presentation type: Poster

Presenting author: Michael Wasserman

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Abstract:

Hormonally active phytochemicals occur across numerous plant species. The most studied hormonally active phytochemicals are phytoestrogens, plant compounds that enhance or block estrogenic activity in vertebrates by binding directly to estrogen receptors and affecting the transcription of estrogen-dependent genes. My research team and I have been studying the prevalence of phytoestrogens in the Uganda red colobus monkey (*Piliocolobus tephrosceles*) diet and its relationship with fecal steroid hormone levels and behavior at Kibale National Park for over a decade using transfection assays to determine the estrogenic activity of plant foods, immunoassays for quantification of cortisol, estradiol, progesterone, and testosterone, and behavioral observations focused on feeding and social behaviors. We conducted an 11-month study of one group of red colobus with data collected from 15 females and 13 males using scan samples and opportunistic fecal collection (n = 990) in 2007-08. We completed a follow-up study in 2017-18 on the same group consisting of one year of focal follows of specific individuals (n = 12) for one continuous month each to collect as close to all feeding behavior and fecal samples as possible for that month. We found that three regularly consumed foods were estrogenic: *Millettia dura* young leaves, *Ficus natalensis* young leaves, and *Eucalyptus grandis* bark. Based on group-level data, there was a significant positive relationship between the percent of diet coming from *M. dura* and both fecal estradiol and fecal cortisol levels in adult males, while data from adult females are currently being analyzed. Data from the focal follow study are also currently being analyzed to test the hypothesis that estrogenic *M. dura* not only alters immunoassay results from fecal samples, but actually has a physiological and behavioral effect. Based on our results, we recommend that researchers consider the effects of dietary hormonally active phytochemicals on physiology and behavior.

Urinary biomarkers of pregnancy and pregnancy-loss detection in the giant panda

Kirsten S Wilson¹, Jella Wauters^{2,3,4}, Iain Valentine⁵, Alan McNeilly¹, Simon Girling⁵, Rengui Li⁶, Desheng Li⁶, Hemin Zhang⁶, Mick T Rae⁷, Alexander F Howie¹, Ruth Andrew¹, William C Duncan¹

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Presentation type: Poster

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Abstract:

Female giant pandas show complex reproductive traits, including diapause, a variable length estrous, and pseudopregnancy, and currently there are no confirmatory non-invasive biomarkers of blastocyst implantation or pregnancy. Urinary estrogen and cortisol concentrations increase during pregnancy in other species, thus we aimed to monitor these across gestation in pregnant, pseudopregnant (non-bred) and non-birth cycles in the giant panda. Urine from 5 pandas (13 estrous cycles) was assessed by ELISA for estrogen (E1G or E1S) and cortisol and normalised against urinary specific gravity. Hormone profiles were aligned to 'Days from PGFM spike', a known fixed point in the cycle, and concentrations were standardised to 'Fold Change of Mean' based on the mean primary rise value for each panda. Statistical analyses were by One-Way ANOVA with Tukey's Multiple Comparisons. A pregnancy-specific profile of estrogen was identified during gestation. Pregnant females showed a 2.2 fold increase of estrogen between the PGFM spike and birth. Urinary cortisol showed a similar profile, with a 3.4 fold increase. Neither hormone showed an increase in pseudopregnancy. This allowed a nomogram, starting at a known fixed point during the cycle, to be created for each hormone, and we assessed how non-birth cycles aligned to these profiles. Non-birth profiles showed deviations from those of success, we believe these may indicate the point of failure of the pregnancy. We believe the urinary estrogen and cortisol profiles are altered in the presence of a fetoplacental unit in late gestation in the giant panda. Both hormones show significantly distinguishable profiles between pregnant and pseudopregnant females in the final two weeks of the cycle. Longitudinal monitoring of estrogen and cortisol after breeding in giant pandas may therefore have the potential to be developed as a much needed panda pregnancy test with the possibility to differentiate between birth and non-birth cycles prospectively.

Non-invasive monitoring of the oestrous cycle of female African lions (*Panthera leo*): can I trust this stool?

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¹ University of Pretoria, Gauteng, South Africa; ² Universidad de Alcala, Madrid, Spain

Presentation type: Poster

Presenting author: Isabel Callealta

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Abstract:

The lion (*Panthera leo*) population counts with less than 30 000 individuals and a decreasing population trend. Ex-situ breeding programs may help to improve the genetic diversity of this species. However, for these programs to be successful, it is a prerequisite to thoroughly understand the reproductive physiology of the species. The aims of this study were 1) to characterize the follicular and luteal phases of the oestrous cycle of the African lioness, by relating its reproductive behaviour to oestrogen and progesterone alterations, and 2) to investigate the usefulness of these endocrine patterns for detecting oestrus/pregnancy when hormone concentrations are determined non-invasively and, thus, sampling is not frequently possible. For 12 months, 5 captive females were observed for signs of behavioural oestrus. In parallel, their enclosures were scrutinized daily, and faecal samples were collected and frozen at -20° C when found. Faecal oestrogen (fEM) and progesterone (fPM) metabolite concentrations were determined in 129 samples from 4 of those females, along 9 months, using competitive biotin-streptavidin enzyme immunoassays against 17 β -oestradiol-17-HS:BSA and 5 β -pregnane-3 β -ol-20-one-3HS:BSA, respectively. Oestrous events included purring, flirting run, lordosis, allowing mount, and rolling. Based on behaviour, follicular oestrous cycles lasted 16.15 ± 1.13 days (n=13; range: 8-23), with oestrus of 6.43 ± 0.47 days (n=14; range: 4-9). Elevated fEM concentrations ($> \text{mean} + 2\text{SD}$) matched oestrous behaviour in 64.29% of cases, but 8 additional peaks out of 17 (47.06%) were found uncorrelated. In all cases, persistent highly ($81.11 \pm 8.53 \mu\text{g/g DW}$; n=45) and moderately elevated ($29.34 \pm 3.85 \mu\text{g/g DW}$; n=28) concentrations of fPM ($> \text{mean} + 1.75\text{SD}$) matched pregnant luteal phases (lasting $108.25 \text{ days} \pm 0.75 \text{ days}$; n=4; range: 107-110) and non-pregnant luteal phases ($49.67 \pm 0.88 \text{ days}$; n=3; range: 48-51), respectively. Infrequent sampling hindered accurate oestrus detection, but allowed identification of luteal phases and distinction between pregnancy and pseudopregnancy.

**Non-invasive monitoring of estrogen and testosterone metabolites in amphibian urine:
Applications for determining gender, seasonal steroidogenesis and spermatogenesis**

Andrew J Kouba¹, Amanda B Gillis¹, Qinju Wang², Beth M Roberts², Carrie K Kouba¹

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Presentation type: Poster

Presenting author: Andy Kouba

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Abstract:

There is very little information on the use of non-invasive monitoring of steroid metabolites to evaluate reproductive function in amphibians. The development and standardization of such techniques for threatened amphibians would be valuable for determining gender in monomorphic species, establishing breeding seasonality and evaluating sperm production in response to exogenous hormone therapy. We proposed that the steroid metabolites for testosterone and estrogen could be measured non-invasively in the urine of male and female adult Fowler toads, as a model species, to answer the above questions. To test this, urine was collected multiple times over 12 hours from individual male (n=14) and female (n=9) toads, pooled and frozen until analysis by an Enzyme-linked immunosorbent assay (ELISA). Urine samples were collected during and outside of normal breeding season as well as following exogenous hormone therapy. For toads treated with the exogenous hormone human Chorionic Gonadotropin (hCG), sperm production was also followed over time for quantity and quality of spermatozoa. We found that both testosterone and estrogen steroid metabolites could be measured in toad urine and that the ratio of testosterone to estrogen was significantly different by gender. Neither female nor male toads exhibited a difference in estrogen production by season; however, both sexes showed a significantly greater production of testosterone during the breeding season. Lastly, both male and female toads showed a rapid rise in both steroid hormones following a treatment of hCG, which overlaid nicely with sperm production in the males. Together, these data show that non-invasive steroid metabolite monitoring in amphibians is possible and could be a valuable tool for monitoring reproductive function, support captive breeding efforts and help sustain living collections.

A combination of non-invasive endocrine monitoring and predictive models to assess pregnancy status in wild guanacos: a population approach.

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Abstract:

The use of non-invasive methods and predictive models is an opportunity to incorporate pregnancy status information on population studies. Usually, this data is not included due to the limitations of pregnancy detection in free-ranging individuals. With the objective of developing a predictive model to diagnose pregnancy status in free-ranging wild guanacos (*Lama guanicoe*), we measured fecal progesterone and estrogens metabolite concentrations in pregnant (n=35) and non-pregnant (n=34) females used for live shearing management (capture, shear and release). Hormonal data was combined with data on abdominal ballottement as an independent factor of pregnancy diagnosis, to develop a logistic regression model which was then applied to assess pregnancy status in unhandled individuals in La Payunia Reserve (Mendoza, Argentina; n=69 females). To build up the predictive model, extracts from dry fecal samples were analyzed to determine pregnanediol glucuronides and conjugated estrogens concentrations by enzymeimmunoassays (PdG R13904 and EC R522-2 respectively, C. Munro). As expected, PdG and EC concentrations were significantly higher in samples obtained from pregnant females under live shearing management (PdG pregnant vs. non-pregnant females: 81.9±58.5 and 45.4±24.4 µg/g respectively, EC pregnant vs. non-pregnant females: 485.6±412.0 and 76.2±47.9 ng/g respectively). The model was applied to hormonal data obtained in free-ranging guanacos to predict percentages of pregnant females in the population (46% in 2016 and 19% in 2017). PdG concentration in free-ranging individuals varies between 15.1-312.0 µg/g in pregnant females, and between 18.8-83.5 µg/g in non-pregnant females. EC concentration extends from 43.6-1119.7 ng/g in pregnant and from 10.6-98.7 ng/g in non-pregnant females. These results will be used to incorporate pregnancy status on guanaco population dynamic studies. Moreover, this study approach might be applicable in other wild species in order to monitor pregnancy in large scale studies.

Use of urinary biomarkers of ovarian function in *Chinchilla lanigera*: assessing protocols for synchronization and ovulation induction.

Verónica I Cantarelli¹, Gabriela F Mastromonaco², Carolina Demaria¹, Magalí Pino¹, Marina F Ponzio¹

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Presentation type: Poster

Presenting author: Marina Ponzio

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Abstract:

Due to the high value of its fur, indiscriminate hunting reduced chinchilla wild populations virtually to the verge of extinction (IUCN critically endangered, CITES Appendix I). A key aspect for the implementation of ex-situ captive breeding programs is the study of the species' reproductive physiology. Our objective was to evaluate the endocrine patterns of ovarian dynamics after its exogenous activation in chinchillas, using luteolytic pre-treatments with prostaglandin-F2 α (PG-F2 α). Two doses of PG-F2 α were tested: Cloprostenol sodium 50 and 100 μ g (Ciclasel-DL); ovulation was then induced using eCG 100 U.I. (Novormon-5000) and hCG 80 U.I. (Ovusyn). Eight females were randomly assigned to each protocol and urine samples collected before, during and a week after the drugs i.m. injection. Samples were analyzed for creatinine (CRT, Sigma #C3613), pregnanediol glucuronide and estrone conjugates (PdG-R13904, EC-R522-2, C.Munro) by EIA. A longitudinal profile was obtained for each hormone and female, using an iterative process to calculate baseline and peak hormonal levels. Although both prostaglandin doses kept progestagen concentrations at basal levels until the application of the inductor (eCG), a more synchronic response afterwards was obtained with the higher PG-F2 α dosis. Using this protocol, 87.5% of the females showed a positive response to eCG at day 8 post-PG followed by hCG at day 13, observing an increase in EC concentration twelve times above baseline levels (0.34 ± 0.06 vs. 4.3 ± 1.03 ng/mg CRT) followed by a PdG peak of fourteen times above baseline levels (153.4 ± 39.9 vs. 2133.7 ± 892.4 ng/mg CRT). EC peak duration was 5.1 ± 0.6 days in coincidence with the natural cycle in chinchillas (6.7 ± 0.9 days). However, PdG peak duration was 3.5 ± 0.5 days, which suggests the occurrence of a very short luteal phase in relation to what occurs in the natural cycle (7.1 ± 0.6 days). Further studies are needed in order to accurately imitate the natural cycle.

Functional relationships between estradiol and paternal care in red bellied lemurs

Madalena O Birr¹, Andrea Baden², Stacey Tecot¹

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Presentation type: Poster

Presenting author: Madalena Birr

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Abstract:

Recent studies have shown increasing evidence to support a neurohormonal basis for paternal care. The role of estradiol in paternal care is relatively unknown, but experimental studies with monkeys and rodents suggest that paternal care requires elevated estradiol. Estradiol levels elevate during a partner's pregnancy, and the transition into fatherhood. However, because estradiol levels are produced by fat tissue, elevated estradiol may be associated with weight gain in fathers rather than paternal care. In this study, we associated estradiol levels in paternal red bellied lemurs (with new infants) with the care behavior that they displayed. We predicted that estradiol levels would predict care behaviors (huddle, hold, groom, carry, play, and a composite score). We measured fecal estradiol in 86 samples from 10 wild red-bellied lemur fathers, and analyzed extracted estradiol metabolites by enzyme immunoassay. We predicted paternal behaviors from mean E2 levels calculated every 4 days. We analyzed data using the R package nmle, with father ID and year as random effects, and the 4-day intervals and paternal behavior as fixed effects. Separate models were run with each behavior and an interaction with interval to represent infant development. Estradiol levels significantly predicted the total paternal care, a composite value of all the behaviors observed, (with significance measured at $P=0.0320$) and specifically huddling ($P=0.0178$). There was also an effect of time on the relationship between estradiol and total paternal care (with significance measured at $P= 0.0453$). These findings support the hypothesis that estradiol levels in red-bellied lemur fathers are associated with paternal behaviors and not merely weight gain.

Ovarian suppression with Improvest[®], a GnRH immunological product in Giraffe (*Giraffa spp*) under managed care

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Presentation type: Poster

Presenting author: Linda Penfold

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Abstract:

Contraception in managed giraffe is challenging, because the goal is frequently dual: population control (contraception sensu stricto) and ovarian suppression (behavior). Males pursue females incessantly during estrus; therefore, frequently recurring estrus year round can be a problem, especially if there is ice on the ground. While effective for contraception in giraffe, porcine zona pellucida, progestins and deslorelin still allow some degree of folliculogenesis and accompanying estrus behavior. Improvest[®] (GnRH immunological product) induces gonadal quiescence by inducing antibodies against endogenous GnRH and minimizing steroidogenesis. The efficacy of Improvest[®] on ovarian suppression was evaluated in seven giraffe. Females were genetically well represented and reversibility was not essential. Treatment consisted of an initial dose (600ug IM, pole syringe/hand injection), booster at 4 weeks and maintenance boosters at 3mo. intervals. Feces collected 3/week and later 1/week were analyzed for pregnanediol-glucuronide (PdG) and estrone-glucuronide (E1Gluc). In cycling females, Improvest[®] was effective at suppressing ovulation and estrus behavior. In all cases the males stopped pursuing the females. Two of three giraffe initially on concurrent oral MGA showed suppressed hormone values prior to treatment. For the remaining five females, cessation of cyclicity was observed, and a GnRH challenge in one female showed no evidence of LH release, indicating suppression. Return to ovarian activity (folliculogenesis and ovulation) was confirmed via hormone monitoring in 3/3 giraffe for which Improvest[®] was discontinued after 15mo, including two that were not cycling prior to treatment. Results indicate short-term Improvest[®] was effective in suppressing ovarian activity, was not associated with clinical adverse effects, and appears reversible for female giraffe. Estrus behavior was occasionally observed after injection of Improvest[®] and adjunct contraception is recommended initially when separation from male is not possible.

Reproductive and adrenal physiology of dwarf mongoose in relation to social changes

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¹ Oregon Zoo, OR, USA; ² Oregon Zoo, Oregon, USA

Presentation type: Poster

Presenting author: Candace Scarlata

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Abstract:

Dwarf mongooses (*Helogale parvula*) are cooperative breeders that live in groups with a dominance hierarchy among same sex individuals. A small colony of this species is kept at the Oregon Zoo. Animal keepers had noticed some increased aggression and possible shifts in dominance among the females. To better understand these changes and help with future management, we decided to monitor reproductive and adrenal hormones. Previous hormone research for this species has focused on urine analyses using RIA assays and found that dominant females had higher estrogen and glucocorticoid levels than subordinates. The objectives of this study were: 1) Validate fecal hormone metabolite enzymeimmunoassays in male and female dwarf mongoose; 2) determine whether social status affects hormone excretion; 3) characterize hormonal changes associated with reproduction and breeding. Fecal samples were collected every other day from 5 (1.4) animals for 5-10 months. For each sample, 0.250g of dried feces was combined with 5 ml 80% methanol, shaken on a rotator overnight, centrifuged for 15 minutes and the supernatant was stored at -20°C until analysis. Female samples were analyzed for fecal progestagens and estrogens and female and male samples were analyzed for glucocorticoid metabolites using EIA techniques. All 5 animals showed a large spike in glucocorticoids the day after the new male was introduced to the group, which provides support for a biological validation of our glucocorticoid assay. Our data support previous findings in which the dominant female showed higher estrogen and glucocorticoid values than subordinate females. In addition, we monitored progestagen concentrations for the first time in this species. Females showed cyclic progestagen changes every 41-51 days. The cycle consisted of 26-30 days of elevated progestagens followed by 14-22 days of low progestagens. These data suggest that dwarf mongooses may be spontaneous ovulators, which is contrary to assumptions published in other studies.

Southern white rhinoceros (*Ceratotherium simum*) transferred from Kruger National Park to New Zealand: a review of reproduction during twenty years ex situ.

Catherine J Morrow¹, Aaron Gilmore², Sarah Jones³, Andrew Gore⁴

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Presentation type: Poster

Presenting author: Catherine Morrow

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Abstract:

In 1999, 12 rhinoceros from Kruger National Park (KNP), South Africa were translocated to Australasia. Two males and four females including one pregnant with calf-at-foot were transferred to Auckland (1:2 plus calf) or Hamilton Zoo (1:2) in New Zealand. Ovarian cycle length, acyclicity and pregnancy were monitored using faecal progesterone concentrations (EIA CL425 and P-HRP, UC Davis, CA, USA). Regular ovarian cycles ranged between 26-38d, a few long cycles of 63-70d were evident and one early embryonic loss was confirmed by ultrasound in a 70d cycle. Temporary periods of anovulation were associated with lactation and seasonal anovulation during autumn/winter months was common in all three captive-born females. Fertile matings (n=14) confirmed by birth or ultrasound, only occurred between August and March with 71% (10/14) coinciding with increasing daylength during spring (September-December). For known full term pregnancies (n=11), median gestation length was 507d and tended to be longer for male (510d, range 500-517d, n=6) compared to female (500d, range 499-510d, n=5) calves. Births were limited to January-June, August with a seasonal peak (8/12; 66.6%) during summer/autumn (February-April). Twelve calves (7:5) have been born to date, 2 females did not survive. As part of the Australasian Species Management Program Captive Management Plan a pregnant KNP female and 4 F1 males have been transferred to Australia and two females (KNP non-parous founder and 1 F1) were transferred from Australia to Orana Wildlife Park. In addition to several pregnancies in Australia & NZ, animal transfers between Auckland and Hamilton Zoos have resulted in i) reversal of long term acyclicity in a KNP female followed by two successful pregnancies ii) regular ovarian cycles and pregnancy in 3 F1 females. One F1 female (12yrs) in her natal herd has failed to breed. Ongoing endocrine monitoring has contributed significantly to successful white rhino management in Australasia.

Non-invasive pregnancy diagnosis via faecal sample analysis in non-domestic equids

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Presentation type: Poster

Presenting author: Sue Walker

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Abstract:

The aim of the current study was to develop an effective non-invasive pregnancy diagnosis method to support ex-situ equid breeding programs using faecal progesterone, oestrogen and/or testosterone metabolite analysis. Faecal samples (n=1176) were collected over five years from pregnant and non-pregnant onager (n=6), Przewalski's horse (n=3) Grevy's zebra (n=3), and domestic horse (n=13). Faecal extracts were analysed using progesterone (P4; CL425) oestradiol (E2; R0008), oestrogen conjugate (EC; R522) and testosterone (TT; R156/7) enzyme immunoassays (EIAs). All data was fitted with a generalized linear mixed model (GLMM) to compare faecal hormone concentrations of cycling and pregnant animals, adding individual as a random factor. Data was pooled by month and trimester to compare samples from multiple individuals. Faecal P4 concentrations remained significantly higher in pregnant individuals from month 5 onwards in all non-domestic species (GLMM, $P < 0.001$). The patterns of faecal E2 and EC concentrations observed in pregnant individuals were more variable between species than faecal P4. Compared to non-pregnant animals, faecal E2 concentrations were elevated for pregnant individuals in trimester 1 and 2 ($P = 0.0218$; $P < 0.001$) for Grevy's zebra; trimester 2 and 3 ($P < 0.001$; $P < 0.001$) for Przewalski's horses; and trimester 3 ($P < 0.001$) for domestic horses. There were no significant differences in E2 concentrations between non-pregnant and pregnant onagers. Preliminary results have demonstrated that faecal TT concentrations rise during equine pregnancy and appear to mirror faecal E2 concentrations. Compared to non-pregnant animals, faecal EC concentrations were significantly higher for the entire duration of pregnancy ($p < 0.001$) in domestic horse; trimester 2 and 3 ($P < 0.001$; $P < 0.01$) for Przewalski's horses; trimester 2 ($P < 0.001$) for Grevy's zebra; and trimester 3 ($P < 0.01$) for the onager. In summary, a rise in faecal P4 concentrations at 5 months of gestation provides a consistent and effective non-invasive pregnancy test for all species tested.

Non-invasive assessment of fecal glucocorticoid and androgen metabolites in pygmy hippopotamus (*Choeropsis liberiensis*)

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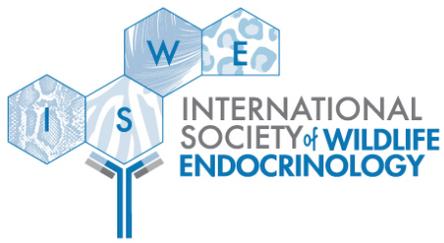
Presentation type: Poster

Presenting author: Gabriella Flacke

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Abstract:

We developed non-invasive methods for characterizing androgen and glucocorticoid profiles in the pygmy hippopotamus (*Choeropsis liberiensis*). Our objectives were to: 1) identify biologically relevant enzyme immunoassays (EIAs) for measuring metabolites of cortisol and testosterone in feces; 2) investigate the relationships among fecal glucocorticoid and androgen metabolites to assess for possible cross-reactions; 3) test for seasonality of androgen metabolite concentrations in males; 4) to test whether gonadal activity in adult males is correlated with breeding status or husbandry practices. For the first objective, we performed an ACTH¹ challenge in 6 animals and then compared several EIAs to assess which produced the most biologically relevant data. Values generated by a corticosterone assay (CJM006, Coralie Munro, University of California, Davis, USA) were significantly correlated with testosterone metabolite concentrations (C196, Arbor Assays, Ann Arbor, Michigan, USA) for both sexes, so this assay could not be used to assess glucocorticoid status. However, two group-specific EIAs exhibiting cross-reactivity with both 11,17-dioxoandrostane (DOA) and 5a-3b,11b-diol metabolites of cortisol reflected adrenocortical activity in both sexes. However, the fecal concentrations of 11,17-DOA metabolites were frequently below the detectable limits of the assay. Thus, we recommend that the 5a-3b,11b-diol EIA be used to study fecal glucocorticoid metabolites in pygmy hippos. The testosterone metabolite assay also produced biologically coherent data: adult males exhibited the highest concentrations, followed by adult females and juvenile males, and proven breeding males had higher concentrations than unproven males. Mean concentrations of androgen metabolites were higher in northern hemisphere spring and summer, and adult males housed outdoors in subtropical climates exhibited higher concentrations than males in temperate climates housed indoors in colder weather. In conclusion, we validated EIAs for fecal androgen and glucocorticoid metabolites that



can be used for monitoring gonadal activity in males and adrenocortical activity in both sexes of pygmy hippos.

Identification and quantification of steroid hormones in polar bear (*Ursus maritimus*) feces by HPLC

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Presentation type: Poster

Presenting author: Kristen Counsell

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Abstract:

Select fecal metabolites have been analyzed using EIA in polar bears, yet the suite of excreted metabolites has not been described. The study's objective was to develop a high performance liquid chromatography (HPLC) method with ultraviolet and/or mass spectrometry detection for the quantification of steroid hormones present in PB feces. Previously quantified values via EIA were used to select male samples with elevated testosterone levels (n=6) and female samples with elevated levels of progesterone (n=3) and testosterone (n=3). Samples were pooled by sex. The spike recoveries from two steroid extraction protocols were compared: 0.2g (+0.002) feces with 2mL of 80% MeOH and 0.1g (+0.002) feces with 3mL of 90% EtOH. Native samples (unspiked sample; NS), solid spiked samples (SSS) and native sample extracts spiked before analysis (NSS) were analyzed. Spiking consisted of 50µg/mL of cortisol, cortisone, corticosterone, testosterone, estradiol (female only), and progesterone (female only). Prior to chromatographic analysis, extracts were cleaned and concentrated using solid phase extraction. All analytes were resolved in 19 minutes with UV detection at 245nm or with a mass spectrometer. The estradiol UV profile was best represented at 280nm. MeOH generated the highest recovery values for glucocorticoids (SSS=0-25%; NSS=42-98%), testosterone (SSS=10%; NSS=71-100%), estradiol (SSS=0%; NSS=91%), and progesterone (SSS=0%; NSS=0%). The following recovery values were from EtOH: glucocorticoids (SSS=0-3%; NSS=36-74%), testosterone (SSS=0-1%; NSS=42-72%), and estradiol and progesterone (SSS=0%; NSS=0%). In males, the following NS hormone values in µg/g feces are: estrogen (87.2), cortisol (14.4), corticosterone (6.62), and progesterone (0.230). In females, the NS hormone values in µg/g feces are: estradiol (52.7), cortisol (9.22), progesterone (7.74), corticosterone (4.48), and cortisone (3.20). In summary, 1) HPLC-UV and LC-MS were validated as a means to quantify PB fecal hormones, and 2) extraction efficiencies are low based on spike recoveries for both methods, but MeOH appears more effective.

Validation of the measurement of progestagen and glucocorticoid metabolite concentrations in feces and analysis through anxiolytic medication administration to facilitate social pairing of an Allen's swamp monkey (*Allenopithecus nigroviridis*)

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Presentation type: Poster

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Abstract:

Allen's swamp monkeys are a highly social primate, often found in large groups. The female Allen's swamp monkey at the Cleveland Metroparks Zoo exhibits stereotypic pacing and heightened aggression toward conspecifics; behaviors interpreted as anxiety-like. Our objective was to validate the non-invasive measurement of fecal progestagen (FPM) and glucocorticoid metabolites (FGM) as indicators of ovarian and adrenal activity, respectively, during administration of two anxiolytic medications, alprazolam (0.039 mg/kg) and ketamine (0.932 mg/kg), and periods of reintroduction to a male conspecific. Fecal samples were collected every other day for four periods: 1) an initial, non-manipulated period prior to drug administration (C1; 4 weeks); 2) administration of alprazolam and introduction to a male conspecific (AZM; 12 weeks); 3) a second, non-manipulated period beginning more than six weeks after AZM terminated (C2; 12 weeks); and 4) administration of ketamine and multiple male introductions (KTM; 12 weeks). Fecal samples were assessed for FPM and FGM concentrations using enzyme immunoassays with antibodies validated in numerous primate species (Progestagens: CL425; Glucocorticoids: R4866) and tested for precision, specificity (by parallelism test), accuracy, and biological validation of FGM through conspecific introduction. Measurement of FPM indicated that this female exhibited a regular elevated pattern ranging from 28-35 days in length (n = 6) throughout all testing periods. Elevated FPM was not different ($P > 0.05$) among periods. Basal FPM and overall FGM, however, were higher ($P < 0.001$ and $P = 0.009$, respectively) during KTM than any other period. The highest concentration of FGM observed (2656.74 ng/g dry feces; 22-fold over baseline) was detected in a sample collected the day after copulation was observed for the first time. Results demonstrate a measurable, physiological response to events experienced during KTM, which indicates support for use of these methods to measure adrenal activity in this species.

Sex determination in Brazilian macaws (*Ara ararauna*, *A. macao* and *A. chloropterus*) by enzymeimmunoassay of progestagen and androgen metabolites from excreta

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Presentation type: Poster

Presenting author: Nei Moreira

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Abstract:

Most Psittacidae species do not present sexual dimorphism, making it difficult to distinguish between males and females. Sexing techniques using DNA have been developed, but usually require blood sample collection, or through feathers, which can be dangerous and/or stressful to the animals. Sexing individuals using non-invasive assessment of gonadal hormones has been successful for some species. In this study, excreta of 17 macaws (13 *Ara ararauna*, 3 *A. macao* and 1 *A. chloropterus*), 10 males and 7 females, from the Municipal Zoo of Cascavel (Municipal Park Danilo Galafassi) were collected (n= 3 samples per animal) for sex determination using validated immunoassays for progestagen and androgen metabolites. All animals had been sexed previously by Polymerase Chain Reaction (PCR). Androgen concentrations did not differ significantly between males and females (66.0 ± 7.3 and 74.2 ± 5.6 , respectively, $P=0.378$); however, progestagen metabolites concentrations and the ratio of progestagen:androgen metabolites were different between the sexes (females, 204.7 ± 36.7 pg/g; males, 90.8 ± 9.4 pg/g, $P=0.0012$; females 3.0 ± 0.3 and males 1.3 ± 0.2 , $P=0.002$). These results show that both concentration of progestagen metabolites and the progestagen:androgen metabolite ratio can be used as a tool for non-invasive assessment of sex in these macaw species. Based on the ratio, birds with a value higher than 2 were considered female, and those under 2 were considered male, representing a 90% accuracy level when compared to the PCR sexing.

Conserving primary antibody stocks: the development of robust indirect competitive enzyme linked immunosorbent assays (ELISA)

Rebecca C Moge¹, Alice S Clark¹, Cayman L Adams², Russell C Hart³, Susan L Walker¹

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Presentation type: Poster

Presenting author: Rebecca Moge

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Abstract:

Enzyme Linked Immunosorbent Assay (ELISA) is a common tool for wildlife endocrinologists to evaluate animal reproduction, health and welfare. The choice of primary antibody (Ab) remains integral to the success of these analyses and therefore, primary Ab made and utilized within wildlife studies is often invaluable and in most cases finite. Capture with secondary Immunoglobulin G (IgG) can be used to decrease the concentration of primary Ab required, conserving valuable primary Ab stocks. Additionally, affinity purified capture IgG specific for the fragment crystallisable (Fc) region of the specific primary antibody is known to have the added benefit of decreasing variability within assay systems. The aim of the current study was to utilise two capture IgGs (affinity purified goat anti-mouse IgG A008 and goat anti-rabbit IgG A009, Arbor Assays) with primary Abs commonly used in wildlife endocrinology research (monoclonal pregnane CL425 and polyclonal corticosterone CJM006, respectively) to develop two robust indirect competitive ELISA systems. The addition of the capture goat anti-mouse IgG (A008; 0.01mg/ml) decreased the required concentration of pregnane Ab CL425 from 1:10,000 to 1:40,000 and demonstrated a comparable sigmoidal-shaped standard curve ($R^2=0.999$). Similarly, the addition of the capture goat anti-rabbit IgG (A009; 0.01mg/ml) decreased the required concentration of corticosterone primary Ab CJM006 from 1:15,000 to 1:50,000 and again produced a comparable sigmoidal-shaped standard curve ($R^2=0.998$). Intra- and Inter-assay coefficients of variation (CVs) were <10% for both high- and low-binding synthetic controls. The above results used ABTS as a system substrate; however, preliminary results demonstrate further dilution of primary Ab is achieved through the use of a more sensitive substrate such as TMB. These techniques can be employed to conserve valuable primary Ab stocks utilized by the wildlife endocrinology community.

Progesterone determination in preserved corpora lutea of bobcat (*Lynx rufus*)

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Presentation type: Poster

Presenting author: Beate Braun

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Abstract:

Post mortem determination of steroid hormones extracted from tissues might be a useful tool to determine hormone activity of reproductive organs in wildlife animals. In the framework of our project on persistent corpora lutea (CL) in lynxes we aimed to use material obtained after hunting to characterize progesterone (P4) production of persistent CL. Often, material of dead animals is fixed in different stabilization reagents for later examinations. The objective of this study was to evaluate whether P4 can be determined in CL that were preserved in Allprotect™ Tissue Reagent (AP). Bobcat carcasses were frozen till sample collection. After thawing, from each animal at least 3 CL of different sizes were collected. One part of each CL (4-50mg) was placed in AP (~500µl), another part was fixed in formalin. After long-term storage, hormones were determined in 54 AP-samples obtained from 12 animals. Homogenized tissue and AP-storage-solutions were extracted two-times with TBME/PE, dried and resolved with 40% MeOH. P4 was analyzed with our in-house EIA using a commercial P4 antibody and 4-pregnen-3,20-dione-3-CMO-peroxidase label. Two pools of samples (tissue and solution) were analyzed by HPLC-immunogram. We were able to measure P4 in extracts of tissue but also in AP-storage-solutions. There was a strong relation of P4 concentration in AP-solutions and the tissue amount/AP-volume-ratio (Spearman's rank correlation coefficient $r=0.6226$, $p<0.0001$). Therefore, for final determination of P4 content of CL tissue, the hormone concentration of the AP-solution must be taken into account too. We could not find a correlation between diameter of CL and P4 content; the mean P4 amount per mg CL was 7.26 ± 6.36 ng. Both HPLC-immunograms showed the main peak at the position of P4. It is possible to measure progesterone in AP-fixed CL but P4 may diffuse into the AP-solution over time. Steroid extraction from formalin-fixed tissue resulted in unreliable results.

Development of a new progesterone metabolite EIA for non-invasive reproductive monitoring

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¹ Arbor Assays, Michigan, USA

Presentation type: Poster

Presenting author: Russell Hart

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Abstract:

Progesterone is a steroid hormone belonging to the progestogen family, regulating female reproductive menstrual cycle, gestation and embryogenesis. The main reproductive hormone progesterone gets metabolized through the activities of enzymes and excreted as a variety of progesterone metabolites in fecal and urine samples. The excreted progesterone metabolite profile will differ from one species to another depending on their unique cellular enzymatic pathways and the length of their gestation cycle. For example, previous reports have shown improved accuracy in monitoring pregnancy when reduced 20-oxo-pregnanes in Black rhinoceros fecal samples were targeted for analysis compared to the parent hormone progesterone. Thus, it is ideal to have more than one approach targeting diverse progesterone metabolites to gain more specific information for each species to aid reproductive strategies. Arbor Assays new kit, K068-H Progesterone Metabolites EIA, offers another method to detect progesterone metabolites that are not theoretically identified by the K025-H Progesterone EIA. The uniqueness of each kit is driven by the cross reactivity of the progesterone antibody in each kit. The Progesterone EIA K025-H uses the CL425 mouse monoclonal antibody, which displays high cross reactivities towards hydroxy progesterones, while the rabbit polyclonal antibody found in new Progesterone Metabolite EIA K068-H shows higher cross reactivities towards reduced pregnane metabolites. In house validation and comparison of K025-H and K068-H using pregnant Maned Wolf urine samples showed different values but similar profiles for pregnancy. However, the validation and comparison of K025-H and K068-H using pregnant Iberian Lynx fecal extracts showed different values and pregnancy profiles for the two kits, suggesting both kits would provide unique and valuable information for the end user to support their research in wildlife endocrinology. We are looking for collaborators to evaluate this assay with a broad range of species.

A novel and highly sensitive non-invasive method to measure steroid hormones in claws reveals sex- and age-specific seasonal variation in cortisol in European Badgers (*Meles meles*).

H Bobby Fokidis¹, Taylor Brock¹, Chris Newman², David W Macdonald², Christina D Buesching²

¹ Rollins College, Florida, USA; ² Wildlife Conservation Research Unit, University of Oxford, United Kingdom

Presentation type: Poster

Presenting author: H. Bobby Fokidis

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Abstract:

Wildlife endocrinology has been greatly enhanced by steroid measures from keratinized tissues, such as hair and feathers. These samples reveal long-term hormone patterns, permitting sampling unaffected by the transient stress of trapping and immobilization which can drastically alter the steroid milieu. We measured claw steroid concentrations in a high-density population of wild European badgers (*Meles meles*) in Wytham Woods, UK. Badgers are strictly nocturnal carnivores inhabiting communal burrows (setts) that are the source of controversial management programs aimed at controlling the spread of bovine tuberculosis. Originally developed for dogs, we sought to validate the claw as a tool for research in badgers by investigating demographic and seasonal variation in four steroids (cortisol, progesterone, testosterone, and dehydroepiandrosterone). We also compared claw steroid levels with established hair sampling methods. Claws were collected during routine trapping, processed using manual grinding, and endogenous steroids purified using solid phase extraction and quantified using enzyme-linked immunoassays (ELISA). Validation experiments for all claw steroids reveal high parallelism between standard and sample curves (all $P < 0.03$); high exogenous recoveries (85.3-96.4%) and increasing steroid extraction with sample mass with optimal recovery around 50 mg (all $P < 0.04$). Claw samples were more precise than hair (% CV: claw 4.8-11.3% and hair 12.6-23.4%), despite high correlations between these two measures ($P < 0.01$). Ongoing research has produced a large sample ($n=409$ nails and $n=301$ hair) and preliminarily has revealed higher claw cortisol concentrations in adult males (8.89 ± 0.09 ng/ml) compared to either adult females (3.36 ± 0.13 ng/ml) or juveniles (3.74 ± 0.18 ng/ml). Furthermore, higher cortisol levels are significantly higher in spring than in the autumn in all groups. Continued research will explore other sources of variation in claw steroids, to develop claw samples as a steroid source for endocrine study and monitoring in mammals that are difficult to study with traditional approaches.

Sex ratios of olive ridley sea turtles in the Pacific: establishing baselines for climate change research

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Abstract:

Sex ratio data provide critical and often underused information to assess the demography and reproductive potential of endangered species, such as sea turtles, with all seven species of turtles listed on the IUCN Red List of Threatened Species. Sea turtle sex is determined by incubation temperature, not sex chromosomes. Warmer incubation temperatures produce more female hatchlings; complete feminization of sea turtle populations is possible under the current climate change projections. Until reaching sexual maturity, sea turtles lack sex-specific external morphology. Therefore, we used blood plasma testosterone (T; ENZO Life Sciences) concentrations to estimate the sex of live immature sea turtles, while tail length (adult males have longer tails) was used to determine the sex of live adult-sized turtles, and gonad visualization was used to determine sex in deceased turtles. This study characterizes the sex ratio of olive ridley (*Lepidochelys olivacea*; vulnerable throughout range) sea turtles foraging in the eastern tropical Pacific (ETP) and Central North Pacific (CNP). We found a male bias (1.9M:1.0F) among foraging adult and immature turtles (n=335) sampled in 2006 during a NOAA Fisheries research cruise in the ETP, yet a female-biased sex ratio (1.0M:2.2F) among olive ridley sea turtles incidentally captured in commercial fisheries in the CNP from 2006–2018 (n=116). Potential explanations for the differences in ratios between the two study groups will be discussed. Overall, these are the first sex ratio data for olive ridley sea turtles foraging in the high seas of the Pacific Ocean and provide a baseline for future examination of sea turtle sex ratio and potential feminization of populations due to climate change. Ultimately, these data will be useful for population viability and sex-based survivorship analyses that can contribute to future conservation measures.

**Endocrine correlates of gender and throat colouration in the Southern Ground-Hornbill
(*Bucorvus leadbeateri*)**

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Presentation type: Poster

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Abstract:

The Endangered Southern Ground-Hornbill (SGH) is group-living, with large territories (100km²) often containing a group of 2 – 12 individuals, including a single breeding alpha pair. The remaining group members, consisting largely of red-throated adult male offspring, assist with rearing, but females are evicted from their natal group once their sexually dichromatic, blue throat colouration develops. However, it has recently been discovered that some individuals displaying blue, originally assigned to be females, were misidentified, as they are in fact males displaying female-like colouration, once assessed using molecular gender assignment. To optimize management practices for reintroductions and captive breeding it is vital that the role of such individuals is understood. An investigation into the suitability of enzyme-immunoassays for urofaecal androgen metabolite (ufAM) monitoring, to characterize changes in ufAM levels related to throat colouration, was developed. Fresh faecal samples were collected from 67 SGHs, of various demographics and origin, across 12 captive institutions, using an entirely non-invasive approach. A comparison of ufAM concentrations of male, female, and juvenile birds showed that sexually mature individuals (> 5 years of age) had significantly higher ufAM levels than respective sexually immature individuals (< 5 years of age) (males: $W=123$, $P=0.0493$; females: $W=262$, $P=0.001625$). There were no significant differences found between adult males bearing blue (B), partially blue (sB) and fully red (R) throats ($F=0.312$, $P=0.736$), however individual median ufAM concentrations of B and sB males were overall >70% higher compared to respective hormone levels for R males. The chosen enzyme-immunoassay has been validated as a suitable method for monitoring ufAM concentrations in SGHs and males with blue throat colouration tend to have higher ufAM concentrations than males with a fully red throat. Therefore, further investigation using ufAM monitoring, with larger sample sizes, is necessary to establish whether a significant correlation exists.

Understanding stress physiology of tigers in the Terai-Arc landscape (TAL), India - a pilot study.

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Presentation type: Poster

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Abstract:

Burgeoning anthropogenic impacts are currently major concerns for the survival of endangered large carnivores globally. Noninvasive physiological measures of psychological stress could prove useful to bridge the intervals between multiple environmental disturbances and their population responses. In this pilot study, we measured stress levels of tiger populations in the Terai-Arc landscape (TAL), India. This landscape retains about 22% of the Indian tiger population across a gradient of undisturbed, high prey, low human use areas in the west to progressively lower tiger density, lower prey and increased human disturbance areas to the east. We collected 250 fresh faecal samples between 2016-18 from Rajaji Tiger Reserve, Lansdowne, Terai West and Central forest divisions in western TAL and Dudhwa and Pilibhit Tiger Reserves in central TAL. Post species-identification through genetic approaches, we measured faecal glucocorticoid metabolites (fGMs) from 150 tiger faeces using corticosterone EIA kits. We conducted parallelism and accuracy tests using randomly selected tiger faeces from study area. At working dilution of 1:100, slope and correlation values were $m = 1.17$, $r^2 = 0.99$. Independent sample Kolmogorov-Smirnov test shows that the population in intact Rajaji-Lansdowne complex ($n=48$) has significantly lower fGMs levels (5.6 ± 3.8 pg/50 μ l), compared to non-protected Terai West and Central forest divisions ($n=31$) (7.6 ± 4.8 pg/50 μ l), as well as from protected but low prey density fragmented habitats of Dudhwa and Pilibhit tiger reserves ($n=51$) (7.30 ± 4.3 pg/50 μ l) in the central TAL ($P < 0.05$). Our preliminary findings indicate that habitat quality and protection status might be significant predictors of physiological stress in tigers. However, further analysis with other ecological variables is required for better understanding of physiological parameters of tigers in this landscape. Such multidimensional approach involving ecological, genetic and physiological measures will be critical for their long-term survival in TAL.

The tail-tale of stress: an exploratory study to assess hair cortisol in Asian elephants

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Abstract:

Assessing cortisol in hairs (hC) has been widely used as a retrospective biomarker for deciphering the 'stressful' past-events in various animals. Such assessments have provided an opportunity for the researchers to collect the minimally-invasive hair samples to understand animals' past physiological state. Being the first exploratory study in Asian elephants, we attempted to assess (i) tail-hair growth rate (TGR) and (ii) hC levels in tail-hair samples from six captive elephants (five females and one male) in the Zoos of Kyoto city (KCZ) and Kobe Oji (KOZ), Japan. The TGR values showed variations among six individuals with a higher rate being in a seven-year-old male (20 mm/month) and a lower rate in a 10-year-old female (10.8 mm/month). For hC analyses, the collected tail-hair samples were either directly pulverized (n = 49 sub-samples from five individuals) or manually trimmed (length \leq their daily TGR; n = 29 sub-samples from two individuals). We employed a validated cortisol enzyme-immunoassay (antibody: FKA404E; Cosmo Bio Co., Ltd., antigen: FKA403) to measure hC levels. We found that hC levels of powdered tail-hair samples, particularly in one of the females-Mito (KCZ), were significantly higher (maximum value = 485.12 ng/g; minimum value = 4.53 ng/g) than her trimmed tail-hair (maximum value = 16.27 ng/g; minimum value = 2.60 ng/g), thereby indicating that the powdered tail-hair samples could be preferred in elephants. Interestingly, when we compared the hC levels of all individuals with the keepers' records, a posteriori, most of the high hC levels were associated with the 'stressful' events (e.g., interactions with other individuals, infection and peculiar behaviours exhibited by them). Our preliminary observations open up a possibility of assessing the hC levels in elephants. However, this approach could be used to assess autopsy tail-hair samples in order to determine the past stress-status of deceased elephants, retrospectively.

Effects of habitat enrichment on stress physiology of Leopard (*Panthera pardus*) under captive conditions

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Presentation type: Poster

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Abstract:

Enrichment of animals' environment improves their physical, physiological and psychological well being. Introduction of enrichment qualities that mimic the natural conditions of the animals will further enhance the improvement. This is particularly true in zoo conditions, where enrichment may allow the animal to perform natural behaviors and may incur lower levels of physiological stress under captive conditions. Thus, to test this hypothesis, we looked at the stress levels of a carnivore species, Leopard (*Panthera pardus*) at the Kamla Nehru Zoological Garden, Ahmedabad, India. Individual leopards were maintained under two different conditions in the zoo: a) Enriched habitat with sound proof glass cages, controlled day-night cycle and controlled temperature; b) Outdoor habitat with open cages maintained under ambient temperatures. All the individuals for both the habitats were kept under similar diet regime. Fecal samples were collected from individuals belonging to both the conditions and were subjected for cortisol metabolite analyses. Preliminary results show that the cortisol levels were not different between the enriched and outdoor habitats ($P > 0.05$), with an average concentration of 0.28 ug/g dry weight across all the individuals ($N=4$ for each habitat type). Further, we are continuing to sample the individuals across seasons to assess the impact of the habitat on the physiology of individuals during more severe conditions, for example, when the ambient temperature reaches 40-45°C during the summer months in western parts of India. Thus, the study will provide insights into the role of captive environment on the well being of an individual and may provide further management implications.

Experimental assessment of feather corticosterone as an integrated measure of hypothalamic-pituitary-adrenal axis activity

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Abstract:

Wild animals are faced with an increasing number and magnitude of stressors associated with our rapidly changing world. Repeated or prolonged stressors can trigger sustained elevations of glucocorticoids, resulting in deleterious effects on health and reproduction. In birds, feather corticosterone (CORTf) is used increasingly to measure cumulative stress responses experienced during feather growth. CORTf is a potentially powerful tool as it may provide historical information on responses to environmental challenges, however a major criticism has been the limited support for the assumption that it reflects blood levels during feather growth. To test this assumption, we surgically implanted CORT or placebo pellets into lesser scaup (*Aythya affinis*) undergoing natural moult. Additionally, ²H-labelled CORT pellets were used to investigate deposition of exogenous CORTf into growing feathers. We measured CORT in serum before, during, and after the active implant period, and in corresponding sections of tail, wing, and back feathers. CORTf was significantly associated with serum CORT for all sections of back feathers, but only for sections emerging from the skin after the active implant period in tail and wing feathers. Endogenous CORT was suppressed by ²H-labelled CORT, which was deposited in all feather sections, including sections grown prior to implantation. Our findings confirm that 1.CORTf may reflect blood CORT levels during feather growth, but the association depends on rate of feather growth, magnitude of stress responses, and length and magnitude of stress responses in relation to time required for feather growth; 2.considerable feather development (hence CORT deposition) occurs within follicles before feathers emerge from the skin, and 3.CORT is deposited throughout the feather until feather growth is completed and/or no longer has a blood supply. This research provides critical evidence to support a central assumption associated with using CORTf, and provides new insight into the physiology of CORT deposition in feathers.

Cortisol, T3, and land use associations in two primate species in Costa Rican lowlands

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Presentation type: Poster

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Abstract:

Native species of monkeys utilize many of Costa Rica's ecosystems, with different anthropogenic and natural pressures in each place. Here we present stress and metabolic biomarkers in primates found in two biological research stations in lowland rainforests contiguous with large national parks with different human activity footprints in surrounding areas. Piro Biological Station is extremely remote and is neighbored by ecotourism hotels, cattle ranches, and Corcovado National Park. La Selva Biological Station is more accessible to cities, highways, and bordered by major banana and pineapple plantations and Braulio Carrillo National Park. To evaluate impact of these human activities, non-invasively collected fecal samples from free-living mantled howler monkeys (*Alouatta palliata*, n=43) and spider monkeys (*Ateles geoffroyi*, n=20) were quantified by enzyme immunoassay for cortisol (Arbor Assays, K003-H1W) and triiodothyronine (T₃, Arbor Assays, K056-H5). There is a significant effect of species (F=17.25, df = 1, 61, P = 0.0001035) and location (F = 6.971, df = 1,61, P = 0.0105) on cortisol values. Specifically, spider monkeys have lower cortisol (95.498 ± 11.490 pg/mg) than howlers (177.23 ± 13.186 pg/mg), and spider monkey cortisol is lower at Piro (70.478 ± 6.534 pg/mg) than La Selva (141.98 ± 21.950 pg/mg; F = 20.07, df = 1, 28, P = 0.0001148). There is also a significant effect of species (howler monkey 507.69 ± 57.291 pg/mg, spider monkey 243.594 ± 49.580 pg/mg; F = 22.14, df = 1, 61, P = 1.499e-05) and location (F = 4.475, df = 1,61, P = 0.03848) in T₃, with spider monkeys again driving the difference through significantly lower levels at Piro (160.63 ± 19.725 pg/mg) than La Selva (397.67 ± 121.28 pg/mg; F = 17.35, df = 1,28, P = 0.0002695). These results indicate that spider monkeys may be particularly vulnerable to human activities, perhaps affecting activity and metabolism.

Capture as an acute stressor: phylogenetic and seasonal variation in avian stress responses

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Presentation type: Poster

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Abstract:

The capture and restraint of wild birds is often necessary in ornithological research but the stress caused by these activities is considerable. Vertebrates respond to stressors by activating the hypothalamic-pituitary-adrenal axis resulting in elevated plasma corticosterone concentrations (pCC) in birds. To quantify the physiological stress response to mist netting and restraint, we measured pCC in three passerines: dark-capped bulbul (*Pycnonotus tricolor*), southern masked weaver (*Ploceus velatus*) and Karoo thrush (*Turdus smithi*), one columbid: laughing dove (*Spilopelia senegalensis*) and one colliiforme: speckled mousebird (*Colius striatus*) during the austral summer. Three of the species, dark-capped bulbul, Karoo thrush and laughing dove were also sampled in winter. The birds were caught in mist nets and blood (0.2 ml) was collected at 3, 5, 10, 20, 30 and 60 minutes for pCC analysis using an antibody against corticosterone-3-CMO:BSA. Both the 3 minutes baseline as well as peak stress-induced pCC varied among the five species, with peak stress-induced pCC occurring at 30 and 60 minutes. Baseline and peak pCC were highest in Karoo thrushes. Speckled mousebirds showed the most pronounced response, an 8-fold increase in pCC. Among the passerines, bulbuls had the lowest baseline pCC and the highest (5-fold) response, whereas thrushes and weavers had a 3-fold increase. All three species had lower baseline pCC in winter than in summer. While the passerine species had higher peak pCC in winter, mounting a 7-fold increase, laughing doves had similar response magnitudes, a 3-fold increase in both seasons. Although all five species show a stress response to capture and restraint, phylogenetic and seasonal differences in both baseline and peak stress-induced pCC were observed. This study provides useful information to bird ringers and researchers on deciding which species to remove first from mist nets.

Seasonal effects of faecal glucocorticoid (fGCM) and triiodothyronine (fTM) metabolites in a model nocturnal strepsirrhine primate population

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Presentation type: Poster

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Abstract:

The dramatic decrease in primate populations globally has been linked to climate change and anthropogenic stressors. As such, it is imperative to study the primate physiological responses to environmental changes to understand primate adaptability and to enhance species conservation strategies. To assess the physiological responses in a prosimian primate to environmental change, we studied the greater thick-tailed bushbaby (*Otolemur crassicaudatus*). Our study had two aims, firstly to validate the most appropriate enzyme immunoassays (EIAs) for monitoring faecal glucocorticoid (fGCM) and thyroid (T3: triiodothyronine) hormone metabolite (fTM) concentrations as biological markers of stress and metabolism; here a handling event and TSH challenge were conducted upon two (1 male; 1 female) *Otolemur* individuals. Next, we examined the effects of seasonality on annual fGCM and fTM patterns of free-ranging male and female bushbabies. We collected samples (male: n = 76; female: n = 110) from free-ranging *O. crassicaudatus* from the Lajuma Research Station, Soutpansberg Mountains. The biological validation (handling event) resulted in a significant increase in fGCM concentration in both sexes (male: 591%; female: 204%), with the cortisol EIA chosen as the most appropriate EIA. Similarly, the TSH challenge resulted in a significant increase in fTM concentrations in both sexes (male: 71,9% increase; female: 67,8%). For females, a mixed effect model revealed the covariate best explaining the variation in fGCM concentrations was season (P= 0,05), while rainfall significantly affected variation in fGCM concentrations among males (P= 0,04). Furthermore, we see a significant difference in ambient temperature between seasons (P< 0,001) which could support our findings. We successfully validated the EIAs necessary for monitoring fGCM and fTM concentrations for *O. crassicaudatus*. Overall, our findings show that changes in the natural environment can have significant effects on the stress and metabolic patterns in this primate species.

Effects of Transfer on Fecal Glucocorticoid Metabolites in Red Pandas (*Ailurus fulgens*)

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Presentation type: Poster

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Abstract:

The endangered red panda (*Ailurus fulgens*) is a favorite of zoo visitors because of its charismatic personality and behavioral antics. In spite of their popularity, red pandas have a reputation of being sensitive to environmental stressors. Like many ex-situ species, red pandas are frequently transferred between institutions due to breeding recommendations; however, their adrenal stress response to relocations has not been previously researched. This study focused on animals transferred between four zoological facilities in the US: Smithsonian's National Zoological Park and Conservation Biology Institute, Seneca Park Zoo, and Evansville's Mesker Park Zoo & Botanic Garden. Fecal samples were collected 3-7 days per week from each individual (n = 5), with collections occurring for up to three months before and after relocation. Samples were dried and fecal glucocorticoid metabolites (fGM) extracted using 80% methanol and a modified shaking technique. Concentrations of fGM were then quantified using a corticosterone enzyme immunoassay (CJM006). Red pandas demonstrated a physiological response to relocation. Both individual variation in baseline fGM concentrations and response to transfers occurred in study individuals. Preliminary results show a variety of responses in fGM concentrations the day after transfer. Red pandas exhibited up to three fold increases, no response, and decreases in fGM concentrations one day post-transfer. These analyses indicate an acute adrenal response to translocation. Examining baseline trends reveal shifts in individual fGM baseline concentrations from the three month pre- to post-relocation periods. Red pandas had either an increase or decrease in their baseline concentration between these two periods. Understanding how red pandas react to stressors, such as relocation, is important for guiding and improving transfer methods to support the long-term health of each animal.

The effects of environmental stress and body condition on African wild dog (*Lycaon pictus*) faecal glucocorticoid metabolite concentrations

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Presentation type: Poster

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Abstract:

Faecal hormone analysis is a minimally invasive method for addressing conservation and animal-welfare questions. Similarly, body condition scoring has been widely used to provide insight into both the dietary ecology and health status of various species. The present study utilizes both of these components in assessing the welfare status of the endangered African wild dog (AWD). Faecal samples were collected from 46 South African AWDs across most of their national range. A competitive enzyme immunoassay utilizing antibody against cortisol-3-CMO: BSA was used to determine faecal glucocorticoid metabolite (fGCM) concentrations. Body condition scores for AWDs were determined through a strict set of visually based criteria, frequently used for domestic dogs and adapted for AWDs, which saw individuals classified as either “underweight”, “ideal condition” or “overweight”. There was no significant effect of body condition, hierarchical status, pack size, sex or age-class on baseline AWD faecal glucocorticoid metabolite (fGCM) concentrations (n=43). Captive AWDs exhibited significantly higher baseline fGCM concentrations than their free-ranging counterparts ($F_{1, 41} = 22.55$, $n = 43$, $P < 0.05$). Body condition scores calculated for free-ranging AWDs yielded two “underweight”, 19 “ideal condition” and two “overweight” individuals, while captive AWDs included two “underweight”, nine “ideal condition” and seven “overweight” individuals. This indicates that AWDs exhibit similar ranges of body condition scores in both captive and free-ranging environments. Although not statistically significant, captive AWDs determined to be “overweight” exhibited median fGCM concentrations approximately 17% greater than “ideal condition” individuals, while free-ranging individuals showed no discernible difference in median fGCM concentrations between “overweight” and “ideal condition” individuals. The present study indicates that captive AWDs experience greater levels of pack instability than free-ranging packs, driving the higher fGCM concentrations found. In addition, the magnitude of the stress response to perceived stressors is not determinable by a purely visual assessment of body condition.

Anthropogenic disturbances and physiological stress in free-ranging primates: a meta-analysis

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Abstract:

Identifying the impact of anthropogenic disturbances on the health, survival, and well-being of species has become a key question of conservation biology. Many primate species are forest-dependent, making them particularly vulnerable to habitat change and excellent ecological indicators in tropical ecosystems. Before affecting primates at a population level, anthropogenic perturbations impact the physiology of individuals. Glucocorticoids (GC) (i.e., stress biomarkers) are metabolic hormones which mediate the energetic demands needed to overcome environmental and social challenges. Prolonged elevation of GC levels may have deleterious impacts on health and fitness by impairing reproduction, growth, and immune system activity of individuals. GC analysis is thus a precious non-invasive tool for assessing stress in wild populations. We present a meta-analysis on the impact of anthropogenic disturbances on adrenocortical activity in primates. We also identify significant confounding factors which influence GC levels, highlight discrepancies in methods and results, and suggest advances for future research. We reviewed all current research comparing GC levels between primates inhabiting undisturbed forest and their conspecifics living in disturbed areas (24 studies, 226 comparisons). Anthropogenic disturbances were classified into five distinct categories: fragmentation, logging, hunting, tourism, and human activity (e.g., mining, urbanization). We ran fixed-effect models to estimate the cumulative effect size of every disturbance category. Effect sizes were estimated by calculating Hedges'd. Our results indicate that fragmentation, hunting, tourism, and human activity had a negative effect with increased GC levels, indicating that primates inhabiting disturbed sites were more stressed than those in undisturbed sites. Only logging had no overall effect. Widespread and extensive human encroachment seems to induce physiological stress responses in primates. However, it is crucial for future research to systematically control for confounding factors (e.g. diet, reproductive status, predatory pressure, seasonality) that may significantly influence GC levels and lead to misinterpretations.

The effect of capture and transport on serum cortisol and total thyroxin concentrations in white rhinoceroses (*Ceratotherium simum*) sedated with either azaperone or midazolam

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Abstract:

Translocation is an inevitable management practice of white rhinoceroses (*Ceratotherium simum*) in Southern Africa and exposes the animals to a variety of stressors. The aim of this preliminary study was to assess the effects of capture and transport on serum cortisol and total thyroxin (TT4) concentrations in free-ranging white rhinoceros bulls translocated within the Kruger National Park for reasons unrelated to the study. Twenty-three rhinoceroses were captured with a combination of either etorphine-azaperone (n=11) or etorphine-midazolam (n=12) and, every two hours during transport, additional doses of azaperone or midazolam, respectively, were administered. Serial blood-samples were collected from an auricular vein at capture (S1), one hour after capture (S2), at the start of transport (S3), and after two (S4), four (S5) and six (S6) hours of transport. Changes in serum cortisol (S1 to S6) and TT4 (S1 and S6) concentrations over time and between treatment groups were compared using general mixed effects models. Serum cortisol concentrations (mean \pm SD) progressively increased from 49.1 ± 18.7 nmol/l at S1 to 131.9 ± 55.1 nmol/l at S4, and decreased thereafter (to 81.3 ± 45.8 nmol/l at S6), indicating HPA axis activation and a stress response. Serum TT4 concentrations (mean \pm SD) were significantly lower at S6 (16.7 ± 4.1 nmol/l) compared to at S1 (21.2 ± 5.1 nmol/l) possibly indicating the chronicity of the stress response. Rhinoceroses sedated with midazolam experienced slightly higher cortisol concentrations towards the end of transport than animals sedated with azaperone; there were no significant differences in TT4 concentrations between the groups. Results indicate that translocation of white rhinoceroses induces chronic stress and alters thyroid-function, which may affect the animal's immune response and ultimately lead to disease and translocation failure. Whether changes in cortisol and TT4 can be used to indicate translocation success, or failure, needs to be determined.

The effects of after-hour visitor events on cheetah (*Acinonyx jubatus*) and giraffe (*Giraffa tippelskirchii* & *Giraffa camelopardalis reticulata*) behavior and adrenal activity at the Oregon Zoo

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Abstract:

Modern zoos increasingly have become centers for entertainment beyond animal viewing. Events held after regular zoo hours, such as concerts, fundraisers, and parties have become core elements of zoo activities, especially during the summer months. Many of these events may expose the resident species to increased noise levels, visitor presence, and prolonged viewing hours. Few studies have quantified the effect of such events on the behavior and physiology of zoo animals. For this study we decided to monitor two species with possible sensitivity to crowds and noise during the 2018 and 2019 summer concert season at the Oregon Zoo (Portland, OR, USA). We monitored behavior using quantitative scan sampling (1-minute intervals for 20-minutes, 3 x evening) and fecal glucocorticoid metabolite concentrations (FGM) (EIA assay; double-antibody method: CJM006) in cheetah (*A. jubatus*) (n=2) and giraffe (*G. tippelskirchi*; *G. c. reticulata*) (n=2) in combination with sound pressure level (SPL dB) measures across 3 months during the following three “treatments”: concert evenings (n=10), other evening events (no concerts, but increased visitors, n=4), and control evenings without any activity (no visitors, n=5). Initial analysis from 2018 showed differences in behaviors across treatment types in cheetahs (ANOVA, p-value < 0.05). Neither giraffe nor cheetah showed a difference in FGM concentrations (Repeated measures ANOVA, giraffe: n=26, $F_{2, 22} = 0.065$, $P = 0.9377$; cheetah: n = 56, $F_{2, 39.22} = 0.1153$, $P = 0.8914$) across treatment type. Additionally, no correlation was found between FGM concentrations and SPL dB (sound) levels (Spearman’s rank: giraffe $S = 32$, $P > 0.05$ & cheetah: $S = 210$, $P > 0.05$). Overall the impact of concerts and other events appeared minor for the study species according to our 2018 results; however data collection will continue during the summer of 2019 to compare outcomes.

Using multiple endocrine indicators to determine acclimation to a zoo-housed setting in white-bellied pangolin (*Phataginus tricuspis*)

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Presentation type: Poster

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Abstract:

The white-bellied tree pangolin in-situ population is rapidly declining due to the illegal trade of their scales as a belief remains they contain medicinal properties. Additionally, their meat is considered a delicacy, and they have limited defenses against humans allowing for ease of capture. Currently, Brookfield Zoo houses 7 of its original imports from 2016 and 2017 and has had 9 births. Observations by animal care professionals show some pangolins appear to adapt well while others experience more health or reproductive challenges. The use of physiological indicators can contribute to assessing and monitoring welfare of this species. The Brookfield Zoo's Endocrinology Lab validated 3 hormone assays (cortisol, DHEA, and aldosterone) to compare the fecal hormone metabolite concentrations between the first few weeks post-arrival to present day (July-August 2019). This preliminary study examines 4 adult pangolin (2.2), one of which was selected as the sole exhibit animal based on perceived positive adaptation to a zoo setting. Biochemical validations were performed on each assay with satisfactory results. A biological validation of the cortisol and DHEA assay was performed on one individual using arrival, quarantine and exhibit transfer data. A single-case AB design randomization test was used due to small sample size ($n=4$) and results show a significant difference ($p < 0.05$) in all 3 fecal hormone metabolites in present day versus arrival time concentrations. Interestingly, the exhibit animal shows overall lower fecal glucocorticoid metabolites (Mean FGM 242.82 ± 216.61 SD) when compared to all non-exhibit individuals (Mean FGM 1130.48 ± 2609.44 SD). This study's long-term goal is to determine the most effective physiological measures to assess white-bellied pangolin welfare and assist in implementation of optimal husbandry techniques.

The effects of pre-hatching elevated corticosterone and post-hatching restrictive food availability on HPA axis development of mallard ducks (*Anas platyrhynchos*)

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Abstract:

Environmental changes (e.g., altered habitat, food supply, climate change, etc.) act as stressors that may impose increased energetic costs in wildlife. Stressors can trigger the hypothalamic pituitary adrenal (HPA) axis to promote corticosterone (CORT) secretion. Increased CORT in laying females may increase deposition of CORT into eggs, which may alter egg viability and offspring characteristics that result in fitness consequences to females and their offspring. Our objective was to investigate impacts of increased CORT in eggs on the HPA axis of developing ducklings. We assessed how in ovo injections of 15 ng/g of CORT (N=24) affected HPA function in mallard ducklings (*Anas platyrhynchos*) compared with sesame oil injected controls (N=26). At six weeks post-hatch, serum CORT was measured at baseline, following restraint, dexamethasone challenge (negative feedback), and adrenocorticotrophic hormone (ACTH) challenge (maximal adrenal capacity). To evaluate responses to a chronic stressor, ducklings were fed a restricted diet at nine weeks for six days, after which baseline and restraint CORT were re-assessed. Serum samples were analyzed by a validated radioimmunoassay. Maximal adrenal capacity did not differ between treatment and control birds (P=0.425). Baseline and restraint CORT concentrations did not differ between treatment and control groups at six weeks of age (P=0.880), or after six days of dietary restriction (P=0.884). Following feed restriction, baseline (P=0.001) and restraint CORT (P=0.001) concentrations were higher than observed at 6-weeks. As samples were obtained 3 weeks apart, elevations may have been associated with feed restriction or growth and development. The dosage of CORT administered did not appear to affect HPA axis development in ducklings. The dose was extrapolated from studies conducted in smaller passerines, given that similar studies have not been done in ducks. Further research is needed to determine effects of maternal CORT on the HPA axis in ducks.

Neurobiology of stress in captive capuchin monkeys (*Sapajus nigritus*)

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Abstract:

Animals have internal needs like humans. If the environment is not suitable for the manifestation of their natural behavior, they may show anxiety or frustration signs. Several scales are used to evaluate the quality of life in medicine and human psychology; however, in zoology, they are not common. This work aimed to evaluate stress and quality of life of capuchin monkeys through behavioral tests and their relationship with stress biological markers. The experiment was conducted in the municipality of Telêmaco Borba, Paraná State, Southern Brazil, using 11 adult capuchin monkeys, including three females and eight males, with ages ranging between two and eight years old. Stress evaluation was estimated through seven serum biomarkers (albumin, lactate dehydrogenase, glucose, C-reactive protein (CRP), blood count, cholesterol, and cortisol), collected from a single sample per animal. The behavioral evaluation was performed from a psychometric scale, adapted from the Pediatric Quality of Life Inventory. The quality of life questionnaire was applied to three zookeepers and included questions related to physical health, stress, and coping, social relations, psychological stimulation, and positive and negative indicators of the quality of life. In this questionnaire, as higher the scale score, indicates worse animal's quality of life. The Heart Rate Variability (HRV), Cardiovagal Index (CVI) and Cardiac Sympathetic Index (CSI) were calculated to study the influence of the autonomic nervous system on the heart and risk of sudden death. There was a significant, positive, strong correlation between the scale and the values of cortisol and CRP of all animals evaluated, with a coefficient of 0.69 and 0.80, respectively ($P < 0.05$); i.e., as the scale increased, cortisol and CRP values also increased. These results may help in the development tools to assess the quality of life, to identify signs of stress in captive capuchin monkeys.

Physiological and behavioural measure of animal welfare in relation to semi-captive African Elephant (*Africana Loxodonta*) interaction programs

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Abstract:

Elephant interaction programs, specifically ones that provide elephant back riding, have come under great public scrutiny within the past decade, as claims have been made that captive elephants are “forced” to participate in these programs and experience “unnatural levels of stress” due to these interactions. Thus far, however, no comprehensive information exists to prove whether these claims are just. We examined the potential impact of human interactions and especially ride-based activities on behavioural and physiological stress-related indicators in African elephants. The study focused on the 15 trained semi-captive elephants housed at a private Game Reserve, South Africa, as well as the free-ranging elephant group(s) roaming under the same ecological conditions in the reserve. Frequent faecal sample and behaviour collection took place over 9 months from both groups and collected faecal material was extracted and analyzed at the Endocrine Research Laboratory, University of Pretoria, using an enzyme immunoassay detecting faecal glucocorticoid metabolites (fGCMs) with a $5\beta-3\alpha\text{-ol-11-one}$ structure. Elephants who participated in elephant-back-safari (EBS) activities showed significant decreases in fGCM concentrations when EBS were discontinued. Similarly, fGCM concentrations of the trained semi-captive individuals who did not participate in EBS also showed decreased steroid concentrations over the same time. Overall, fGCM concentrations of the trained semi-captive herd and the free-ranging herd did not differ significantly. EBS participating elephants and non-EBS participating elephants demonstrated very similar behavioural patterns throughout the entire monitoring period, and followed similar patterns observed in other populations of free-ranging as well as captive elephants. The collected data on physiological stress levels of trained semi-captive elephants during and post participation in EBS will help to better understand the effect of direct anthropogenic interactions on behavioural and endocrinological stress-related markers of elephants. This approach will aid in efforts to optimize welfare and safety management for semi captive elephant populations.

Non-invasive monitoring of adrenocortical activity in free-ranging Namaqua rock mice (*Micaelamys namaquensis*) in response to anthropogenic land use and season

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Abstract:

Stress in animals is linked to behavioural and physiological changes in response to environmental, social and anthropogenic stimuli. Hence, stress-related responses in animals, specifically in pioneer species such as rodents, are used as biological indicators of ecosystem health. As a prerequisite, this study aimed to establish an enzyme immunoassay (EIA) for monitoring stress-related endocrine correlates in Namaqua rock mice using faeces as a matrix. Subsequently, the established EIA was used to assess the effects of land use practices and season on faecal glucocorticoid metabolite (fGCM) concentration. Rodents were live-trapped seasonally across four land use types: an agricultural crop farm, an agricultural livestock farm, a human-populated peri-urban area, and a nature reserve; all situated in Limpopo Province, South Africa. An EIA detecting steroids with a 5α - 3β - 11β -diol structure was biologically validated using fGCM concentrations at capture and recapture events ($n = 9$). A significant overall 40% elevation of individual fGCM concentrations was found, thus demonstrating the effectiveness of the chosen EIA to reliably detect glucocorticoid output in the study species. Land use type and seasonal interactive effects showed no significant differences in fGCM concentrations, however, the highest median values were recorded in the dry season. This variation between seasons, even within agricultural landscapes offering year-round food availability, suggests that environmental factors such as rainfall and climate can induce stress in small mammals. To ensure that *M. namaquensis* continues to thrive despite anthropogenic disturbances and climatic variation, continuous research into their behaviour in both pristine and altered environments is required. As the species is resilient and adaptive, any significant stress hormone differences could indicate an unhealthy ecosystem and signal indigenous species collapse.

Establishing a non-invasive method for monitoring adrenocortical function in roan antelope (*Hippotragus equinus*)

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Abstract:

Roan antelopes (*Hippotragus equinus*) are popular game ranching herbivores in southern Africa, with increasing numbers in captivity over the last decades. In this regard, animal welfare, especially the avoidance of stress, is nowadays recognised to be a crucial component when managing wildlife. A non-invasive approach to assess adrenocortical function as a measure of stress would be preferable, as animals are not disturbed during sample collection, and therefore sampling is feedback free. So far, however, a non-invasive method has not been established for Roan antelope. As an initial step, we therefore performed an adrenocorticotrophic hormone (ACTH) stimulation test, using an adult male and female individually housed at Lapalala Wilderness, South Africa, to determine the stress-related physiological responses. In total 86 faecal samples (male: 46, female: 40) were collected and the resulting faecal extracts were analysed for immunoreactive faecal glucocorticoid metabolite (fGCM) concentrations using five enzyme immunoassays (EIAs); (i) 11-oxoetiocholanolone I (detecting 11,17 dioxoandrostanes), (ii) 11-oxoetiocholanolone II (detecting fGCM with a 5 α -pregnane-3 α -ol-11one structure), (iii) a 5 α -pregnane-3 β -11 β ,21-triol-20-one (measuring 3 β ,11 β -diol CM), (iv) a Cortisol and (v) a Corticosterone. All EIAs detected increases in fGCM concentration 100% post-ACTH administration for both animals. However, the 11-oxoetiocholanolone I EIA performed best, with a 20-fold increase in the male (baseline: 0.384 μ g/g, DW; peak: 8,585 μ g/g DW) and a 17-fold in the female (baseline: 0.323 μ g/g DW, peak: 7,276 μ g/g DW), measured 17 hours and 12 hours post-administration respectively. The ability to assess adrenocortical function non-invasively in Roan now provides an essential prerequisite to putative stressful circumstances in this iconic antelope species.

Stress and reproductive hormone profiles as measured in baleen from two humpback whales (*Megaptera novaeangliae*) of different sex and habitat

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Abstract:

Humpback whale populations are slowly growing after decimation from commercial whaling. Although their numbers are increasing, little is known about their reproductive cycles or stress patterns because of their cryptic underwater life histories. Our objectives were to determine differences in glucocorticoids and testosterone between two adult humpback whales of different sex and habitat. Study objectives include analysis of one female and one male humpback for cortisol, corticosterone, and testosterone. The adult female was from the Atlantic population, eleven years old at death, and was entangled during the last portion of her life (approximately nine months). The male humpback was from the Pacific population, 66 years old, and died from a long-term chronic illness. Cortisol, corticosterone, and testosterone were quantified with an enzyme immunosorbent assay with subsamples taken along every 2 cm along each plate. Samples were drilled from the baleen plate, the powder was collected, and hormones were extracted using a methanol solution. Cortisol and testosterone were found to be significantly higher during the female's entangled period ($P < 0.05$) but corticosterone was not significant, which suggests that the two glucocorticoids might respond differently to certain stressors. The male humpback showed an increase in stress hormones over the growth of the baleen plate (approximately 4 years of growth), peaking at 6 months before death when the glucocorticoids decline rapidly. Interestingly, testosterone shows an opposite trend, increasingly dramatically to 3.8 times the average during the last six months of life (linear regression for cortisol and corticosterone, $P < 0.001$). Additional investigation of glucocorticoids and reproductive hormones in baleen could prove useful for determining long-term, multi-year patterns in stress physiology of large whales, retrospectively analyzed from stranded or archived specimens.

Comparing the performance of “native hormone” versus “group-specific” assays for assessing faecal glucocorticoid metabolites in African herbivores

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Abstract:

Endocrine markers provide an assessment of individual responses to environmental challenges that ultimately impact on whole-organism function including metabolism, thermal regulation, nutrition, reproduction and immunity. Non-invasive measures are ideal for understanding the physiological state of an animal without causing perturbations by handling or sampling. However, given the changes native hormones undergo during metabolism, excretion and from the microbiome, applying off-the-shelf native hormone EIA's to faecal material is not straightforward and may not be the best choice. None-the-less, native hormone EIA's have been promoted for general use in wildlife studies. Directly comparing the responses measured by native hormone assays with those of group-specific metabolite assays during mandatory validation studies provides an excellent opportunity to assess the impact of assay choice on the inferred stress response. Here we review faecal glucocorticoid metabolite studies across African herbivores and highlight a gap in robust (physiological/biological) validations for some assay/species combinations. We also present validation data from large herbivore species (horses, Cape mountain zebra, Grevy's zebra, black rhinoceros as well as previously published data from giraffe) where native hormone and metabolite assays have been directly compared across samples. In these species, biological validations of native hormone and group-specific metabolite assays are poorly correlated and provide very different pictures of individual stress responses. In all species, metabolite assays showed a pronounced response, whereas the native hormone assays failed to consistently identify a stress response across individuals. This lack of consistency raises the potential concern that using assays that are not measuring the intended molecules may at best be uninformative, or at worst, provide misinformation. When applied to endangered species conservation, using inappropriate assays to assess population health, guide management practices and predict future responses, can undermine conservation activities and potentially lead to detrimental impacts.