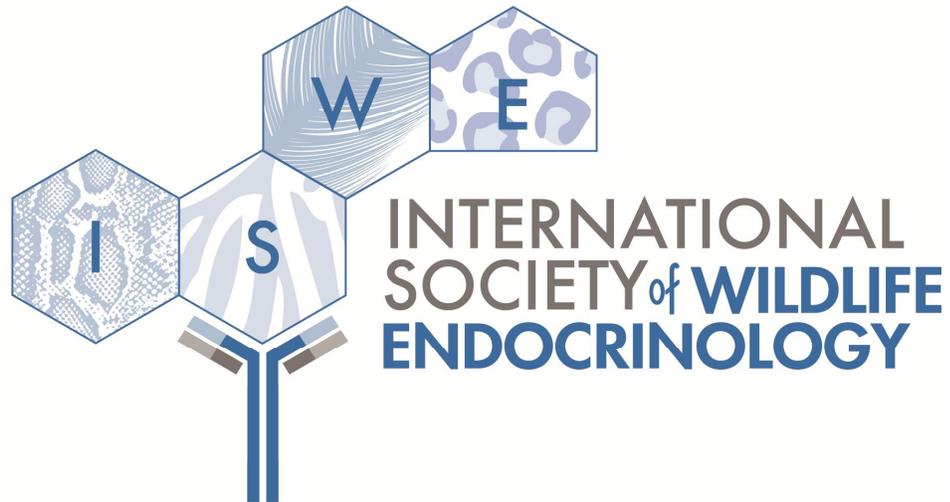


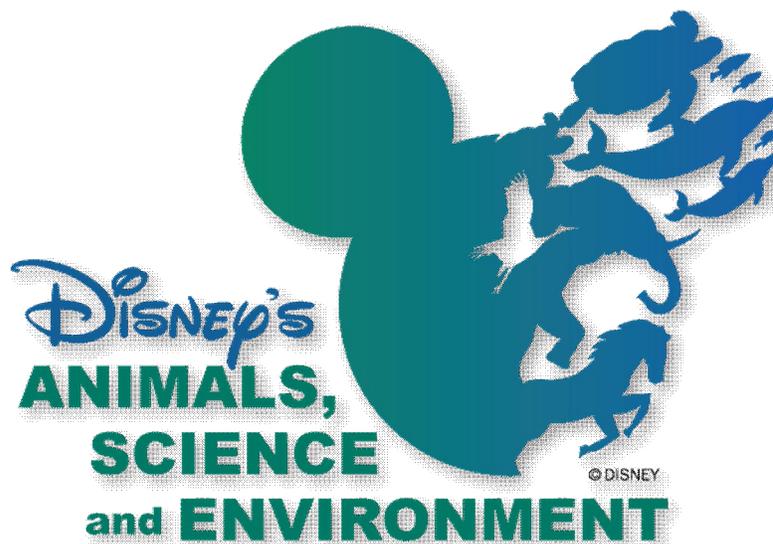
PROCEEDINGS

of the 6th ISWE CONFERENCE

14-16 August 2017 - Orlando, FL, USA



Hosted by Disney's Animal Kingdom



Welcome to the 6th Conference of the International Society of Wildlife Endocrinology!

Welcome to the 6th Conference of the International Society of Wildlife Endocrinology, held this year at Disney's Coronado Springs Resort in Orlando, FL from 14-16 August, 2017.

We have had a busy and productive 2 years at ISWE since our last conference, including website updates www.ISWE-endo.org, automatic membership payment online and a membership renewal reminder system, obtaining official nonprofit status in the US, establishing a 5-year strategic plan, forming official communications, membership, conference and antibody development committees, and launching the Wildlife Endocrinology Information Network (WEIN). Stay tuned for our annual membership update at lunch on the second day of the conference to learn more!

We would like to thank outgoing board members Elizabeth Freeman and Ned Place, as well as Dave Kersey, who graciously agreed to stay on the board as Treasurer for an extra year due to an unforeseen event that prevented last year's voted treasurer from joining the board. They have volunteered their time over the past 3-4 years and have been extremely productive during their time on the board. They will finish their terms at the end of 2017.

This year, 78 abstracts were submitted and 72 were accepted for presentation and inclusion in the conference proceedings. We had 22 colleagues reviewing manuscripts and abstracts this year and we would like to thank them for their time and effort in the review process. Also, we received 16 very competitive submissions for student travel awards. We thank Arbor Assays for sponsoring a \$1000 travel award, and due to their generosity, we were able to award three travel scholarships this year.

In addition to our normal focus on reproduction, health and welfare, we are happy to have a special sessions this year focused on aquatic species, a growing area for study.

With our focus on aquatic species, we are very excited to have our two keynote speakers, Dr. Tyrone Hayes of UC Berkeley, and Dr. James Gelsleichter from the University of North Florida, who will be presenting talks focused on endocrine issues in amphibians and elasmobranchs.

We thank all the speakers and poster presenters, for sharing new techniques and technologies and providing new insight into endocrinology in wildlife. We had an earlier abstract deadline than usual to accommodate our August conference date and we appreciate everyone's patience and willingness to work hard to meet deadlines.

A very special thanks goes to our Conference Host, Catharine Wheaton of Disney's Animal Kingdom and Conference Chair, Marina Ponzio, who have worked tirelessly behind the scenes for many months to organize what we hope will be a productive and entertaining conference!

Finally, we thank you all for attending the conference. We are a small but growing community and it is through these conferences that we learn how to better study wildlife and embark on collaborative ventures. If you are new to the group do not hesitate to reach out to colleagues to ask them about what tools and techniques they have used in various species. We are a hugely collaborative group and that is one of the things I cherish most about ISWE. It has been a pleasure to serve you and our society as the Board of Directors. We encourage you to engage in stimulating discussions during the conference and welcome new members into our group.

We hope you all enjoy your stay in Orlando!

Mandi Wilder Schook, ISWE Chair

ACKNOWLEDGMENTS

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

Janine Brown	Smithsonian Conservation Biology Institute, Front Royal, VA, USA
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Andre Ganswindt	University of Pretoria, Pretoria, South Africa
Katarina Jewgenow	Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
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Rachel Santymire	Lincoln Park Zoo, Chicago, IL, USA
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Catharine Wheaton	Disney's Animal Kingdom, Bay Lake, FL, USA
Mandi Wilder Schook	Cleveland Metroparks Zoo, Cleveland, OH, USA
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Veronica Cantarelli	Facultad Ciencias Medicas, Univ. Nacional de Cordoba, Argentina
Franz Schwarzenberger	Dept. of Biomedical Sciences, Univ. Veterinary Medicine, Vienna, Austria
Carrie Vance	Mississippi State University, MS, USA
Jonathan Aaltonen	Omaha's Henry Doorly Zoo and Aquarium, Omaha, NE, USA

Our Conference Planning Subcommittee

Catharine Wheaton (Host)
Marina Ponzio (Conference Chair)
Lara Metrione
Cayman Adams
Edward Wilkerson
Rachel Santimyre
Shana R Lavin
Katie L Edwards
Meredith Bashaw

We warmly thank our invited speakers for contributing keynote lectures:

Dr. Tyrone Hayes - Department of Integrative Biology, University of California, Berkeley.
FROM SILENT SPRING TO SILENT NIGHT: A TALE OF TOADS AND MEN.

Dr. James Gelsleichter - Department of Biology, University of North Florida.
NONLETHAL APPROACHES TO ASSESS SHARK AND RAY REPRODUCTION IN THE FIELD.

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PLENARY SPEAKERS



Dr. Tyrone B. Hayes is a biologist, herpetologist and professor of the Department of Integrative Biology at the University of California, Berkeley.

His research focuses on the role of steroid hormones in amphibian development including growth, metamorphosis, sex differentiation and hormonal regulation of aggressive behavior. He conducts both laboratory and field studies in the U.S. and Africa and currently, his work also examines the effects of exogenous steroids on gonadal differentiation and the potential role of endogenous steroids. His main goal is to understand how an animal translates changes in its external environment to internal changes, how these internal changes are coordinated, what molecular mechanisms are involved, and in turn, how changes at the molecular level affect an animal's ability to adapt to the changes in its external environment.

Dr. Hayes became well known for his research findings concluding that the herbicide atrazine is an endocrine disruptor that feminizes male frogs, warning the scientific community about how synthetic chemicals that interact with hormones can alter developmental responses, and play a role in the global amphibian decline, and how these studies predict effects in humans and wildlife.



Dr. Gelsleichter is an Associate Professor of Biology in the Department of Biology at the University of North Florida.

His research programs focuses on three major topics: the ecology of shark populations in northeast Florida waters, the reproductive biology and physiology of shark and their relatives and the effects of environmental pollutants on sharks and other fish species.

These three research areas are highly interrelated and research is also integrated with the study of how hormones regulate various aspects of shark reproduction.

Finally, his research also expands to the study of shark populations and other fish exposed to environmental pollutants such as mercury, industrial chemicals and oil that are experiencing health effects that could impair their reproduction, survival, and overall population stability.

WORKSHOPS

INTRODUCTION TO QUANTITATIVE ANALYSIS OF WILDLIFE HORMONE DATA USING R.

Leaders: Pete Laver, Mandi Schook.

This will be an interactive workshop designed to provide researchers an entry into quantitative analysis of hormone data using the R language and software platform. We will discuss various quantitative approaches specific to wildlife endocrinology as well as more general approaches to statistics.

We will interactively step through some basic analysis of hormone data using R and RStudio, and briefly work together on topics such as descriptive statistics (including frequency distributions), power analysis and study design, frequentist statistics (parametric and nonparametric), Bayesian statistics, and information theoretic approaches. Participants should come away with a better understanding of the general approaches they can take, an improved quantitative vocabulary for discussing their data with a statistician, and the tools necessary for finding further information and help. All participants will be expected to bring their own laptops with relevant software pre-installed (instructions for this will be sent to participants in the weeks prior to the workshop).



ASSAY VALIDATION: IDENTIFYING AREAS OF UNCERTAINTY IN CONDUCTING VALIDATION STUDIES.

Leaders: Meredith Bashaw, Janine Brown, Rupert Palme, Kerry Fanson.

Proper validation of an assay is essential in order to gain meaningful insights from excreted glucocorticoid metabolite (GCM) data. However, anyone who has conducted a validation study has likely been faced with challenging decisions about experimental design, assay selection, and data analysis.

These challenges are exacerbated by the fact that many of us are dealing with sensitive species in zoos or in the wild. Although there are many papers that discuss the importance of validation and some of the factors to consider, there are still several aspects of the validation process where questions abound. The purpose of this workshop is to identify key questions and uncertainties about conducting validation studies, with a focus on the assay of GCM in urine and feces. We will draw on case studies, published papers, and personal experience to understand how different methodological approaches can influence the outcome or interpretation of a validation. Participants will be asked to identify and rank questions they have about validation studies with the aim of highlighting key knowledge gaps, from sample collection through to data analysis. This workshop is the first part of a long-term project to develop a set of well-researched guidelines, backed by empirical evidence, about the do's and do not's of validation studies.



WORKING WITH WEIN.

Leaders: Edward Wilkerson, Rachel Santymire.

The Wildlife Endocrinology Information Network (WEIN) is a web based, searchable data network containing information on the endocrine methods and analyses including sample type, sample processing, hormone assays and endocrine results on various domestic and wildlife species both in situ and ex situ. The database allows the sharing of information regarding endocrine monitoring in a given species and is a reference place for endocrinological techniques and species based information.

The goal of WEIN is to become the primary resource for researchers worldwide to learn, analyze, and share information about the growing field of wildlife endocrinology. WEIN will provide a venue for techniques and data that are not publishable, creating a critical resource for people interested in zoo and wildlife health and conservation.

In this workshop, you will be introduced to WEIN. We will take you step by step on how to search and extract information, entering and submitting a project and reviewing a project. Finally, we will discuss the future of WEIN and how it can evolve with the needs of our membership.



TRAVEL GRANT AWARDEES



ARBOR ASSAYS



Sanjeeta Sharma Pokharel
PhD Student
Indian Institute of Science
India

Sanjeeta Sharma Pokharel is a PhD student at the Centre for Ecological Sciences, Indian Institute of Science. She is working to understand the physiological manifestation of the stress-response in free-ranging Asian elephants in the forests of Southern India after beholding intense elephant-human conflicts and the paucity in our understanding about how these adverse perturbations in ecologically modified landscapes could influence the health and welfare of elephants. The first of its kind in the context of free-ranging Asian elephants, her research focuses on the influence of ecological and anthropogenic factors on the stress status of elephants in the forested areas and the fragmented landscapes, aiming to gain sufficient insight to create better conservation strategies. As one of the few researchers who have overcome the challenge of working with elephants in areas of conflict, she hopes that findings from her research will make a significant contribution towards our understanding of stress-physiology and conservation of Asian elephants.



Coralie Munro Memorial



Gabriela Martins
PhD Student
University of São Paulo
Brazil

Gabriela Martins graduated from São Paulo State University in Veterinary Medicine in 2012. Her first research involved validation of enzyme immunoassay for progestins and androgens in droppings of blue-fronted amazon parrots. She then joined the Deer Research and Conservation Center for a Master's degree in Animal Reproduction investigating the existence of reproductive isolation among two sister species of grey deer by assessing hybrid males. She is now a PhD student in Animal Reproduction at the University of São Paulo, and her research aims to establish a chronology of endocrine function to gain a better understanding of the secretory patterns of steroids across life history stages of the maned wolf. In order to help the endocrine interpretation and future ecological and reproductive assessments, the research also intends to develop an epigenetic age assay for the species using DNA of both blood and fecal origins.



Kristen Counsell
M.S. Student
Mississippi State University
United States of America

Kristen Counsell is a Graduate Research Assistant working towards a Master's degree in Animal Physiology at Mississippi State University (MSU). Her thesis investigates a non-destructive, holistic and relatively new approach to determine animal physiologic states including stress, estrus, and pregnancy from biological fluids. This unique technique, referred to as aquaphotomics, is the study of aqueous solutions by near infrared spectroscopy. Her primary goal is to develop and grow into a strong and engaging leader alongside her colleagues, as well as a mentor to younger, aspiring scientists. Currently, Kristen supervises six undergraduate science majors working at the MSU amphibian research facility. Earlier this year, Kristen was inducted into the College of Agriculture and Life Sciences' Outstanding Graduate Student Hall of Fame for her exemplary research, leadership, and collaborative work at the local and national level.



Abstract #38

“One-Shot” immunocontraception for population control in free-ranging Capybara – a South-American mammalian species - *Hydrochoerus hydrochaeris*

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Presenting and contact person: Rosenfield DA, dro@usp.br

Topic: Reproduction

Presentation type: Poster

Abstract:

Human-Wildlife-Conflicts, an increasing and sad reality around the globe, making wildlife and feral population control a multi-facet challenge in wildlife management. For decades, wildlife species, mainly in confinement, have undergone fertility control, largely hormone-based contraceptives, however, in free-ranging animals, challenges are unique, and the demands on contraceptive methods differ. A proposed “perfect” contraceptive would be: Highly effective; long-term action; reversible; little pathological effects; minute behavioral impacts; applicable in both genders; remote delivery (avoiding stress); “one-shot” administration (no booster); no risks of becoming a pollutant; and deemed humane. During 20 years of research, immunocontraception, specifically PZP, - and anti-GnRH vaccines have proven successful in controlling wildlife population, offering most of the desired characteristics. Capybaras, world’s largest rodent (*Hydrochoerus hydrochaeris*), are highly proliferative and extraordinarily resistant to environmental influences. Lacking natural predators, and an overabundance of food, they rapidly reach pest-like population niveaux in Brazil, provoking human- “capybara”- conflicts: urban invasion; traffic accidents; crop destruction; and promoting the tick-spread zoonotic disease Rocky Mountain Spotted Fever. We attempted to trigger an auto-immune response with a single anti-GnRH vaccine, resulting in the reduction of sex hormone synthesis, consequently, provoking infertility in both genders. Prior to vaccination, biometrical and hormone references were established in free-ranging capybaras, n=2♀: 1Test/1Sham (TG/SG); afterward, inoculated with the anti-GnRH vaccine, and a sham vaccine. 27 days post-vaccination, the first analysis of serum estrogen (E2) and progesterone (P4) were conducted, employing non-species-validated commercial radioimmunoassay kits. Pre-vaccination: \bar{x} serum estrogen 1262.47pg/ml and progesterone 22.9ng/dl. Post-vaccination TG: E2 773.97pg/ml vs. SG: 1218.46pg/ml, and TG: P4 22.9ng/ml, vs SG: 34.4mg/ml. The preliminary findings resemble contraceptive effects within one month, post-vaccination, although suggesting a potential as an effective antifertility treatment, the complete study result is required to validate contraceptive activity in free-ranging capybara.



Abstract #40

Linking plasma sex-steroid levels to the condition of external genitalia in European badgers (*Meles meles*): A critical evaluation of traditional field methodology

¹Sugianto NA, ¹Buesching CD, ²Malo A, ¹Macdonald DW

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Topic: Reproduction

Presentation type: Oral

Abstract:

Field biologists often rely on external genitalia examination as a proxy for reproductive condition. In seasonally breeding badgers, scrotal testes are used as an indicator of spermatogenesis, while a pink swollen vulva with mucosal secretion is assumed to indicate oestrus. Systematic validation of these assumptions is lacking. Enzyme-Immuno-Assays were used to compare circulating plasma testosterone (anti-testosterone-R156/7) levels in males (n=103); oestrone (polyclonal-EC-R522-antiserum; n=59), oestradiol (oestradiol-17-HS-BSA-antiserum; n=36) and progesterone (monoclonal-CL425-antiserum; n=61) levels in females with concurrent genitalia condition (males: testes descended, intermediate, ascended; females: vulva swollen/moist, flat/dry). A single blood sample/individual was collected from wild badgers under sedation during winter mating season, spring post-weaning period, summer secondary mating peak, and autumn reproductive quiescence; genitalia condition was categorised according to established field protocols. In females, oestrone was low throughout the year, reaching a summer minimum (32.62 ± 6.32 pg/ml, n=12, $p < 0.001$), higher levels in spring (60.92 ± 3.77 pg/ml, n=23), a peak in autumn (87.94 ± 3.27 pg/ml, n=9, $p = 0.022$) and remaining elevated for winter pregnant females (82.55 ± 14.56 pg/ml, n=10, $p = 0.064$). Oestradiol was low throughout the year (26.85 ± 3.54 — 44 ± 6.12 pg/ml), albeit significantly higher in spring (117.85 ± 38.02 pg/ml, n=13, $p = 0.008$). Progesterone was low throughout seasons (0.32 ± 0.07 — 0.47 ± 0.03 ng/ml), but increased significantly during winter pregnancy (1.34 ± 0.11 ng/ml, n=11, $p < 0.001$). Male testosterone was high in spring (2.06 ± 0.24 ng/ml, n=29) and summer (1.27 ± 0.11 ng/ml, n=33), dropped in autumn (0.81 ± 0.13 ng/ml, n=28, $p = 0.002$) and peaked during winter mating (4.83 ± 1.15 ng/ml, n=13, $p < 0.001$). Our results confirm testosterone and oestradiol are important for mating, oestrone has regulatory function during delayed implantation, and progesterone governs implantation and pregnancy until parturition. Although sex-steroid levels exhibit seasonal patterns, broadly reflecting seasonal variation in genital condition on population-level, on an individual level no relation was found between circulating testosterone and degree of testes descent ($F_{2,98} = 0.505$, $p = 0.605$), nor between oestrogens and vulva condition (oestrone: $F_{1,50} = 2.872$, $p = 0.096$; oestradiol: $F_{1,21} = 1.188$, $p = 0.288$). Thus, extrapolating physiological reproductive status from external genitalia condition needs to be taken under advisement.



Abstract #42

Ovarian fecal hormone metabolite monitoring provides evidence of induced ovulation in the maned wolf (*Chrysocyon brachyurus*)

¹Jones MK, ²Reiter LE, ³Gilmore MP, ³Freeman EW, ¹Songsasen N

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³School of Integrative Studies, George Mason University

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Topic: Reproduction

Presentation type: Oral

Abstract:

To better understand induced ovulation in the maned wolf (*Chrysocyon brachyurus*), 44 reproductive cycles (2002-2016) of 33 females were studied relative to reproductive status. Six months of fecal samples were collected each cycle, with approval from the Smithsonian's Institutional Animal Care and Use Committee. Hormone conjugates were extracted from lyophilized samples using shaking (30 min at 70 RPM) in 90% ethanol. Fecal estrogen (FEM), progesterone (FPM), and cortisol (FCM) metabolites were quantified using single antibody enzyme immunoassays for estrone conjugate (polyclonal antibody R522-2), pregnane (monoclonal antibody CL425), and cortisol (polyclonal antibody R4866) across three estrous cycle phases: pre-ovulatory, peri-ovulatory, and luteal. Reproductive status was classified as: pregnant and raised pups (n = 11), pregnant but neonatal pup demise (n = 7), paired with male but no signs of breeding (n = 10), housed singly (n = 8), housed with related females (n = 7), or housed with a castrated male (n = 1). Females housed without an intact male showed significantly lower concentrations of FEM (183.0 ± 12.8 ng/g feces) and FPM (4.9 ± 0.3 ug/g feces) than females with an intact male (FEM = 226.4 ± 9.0 ng/g feces; FPM = 34.6 ± 1.3 ug/g feces) (FEM: $t_{985} = -12.21$, $P < 0.0001$; FPM: $t_{1238} = -32.12$, $P < 0.0001$). Based on previous publications, ovulation was defined by a mean luteal FPM concentration above 10ug/g feces. Using this definition, 27 of 28 females housed with a reproductive male ovulated while only 2 of 16 females housed without a reproductive male ovulated, providing concrete evidence of ovulation induced by cues from the male. The female housed with the castrated male showed no elevations in either ovarian hormone, suggesting ovulation is not induced via physical cues. The mechanism of ovulation induction in this species is suspected to be testosterone-dependent male chemosignals.



Abstract #43

Determining the efficacy of dopamine agonist, Cabergoline, and dopamine antagonist, Domperidone, for treating ovarian acyclicity in zoo African elephants (*Loxodonta africana*)

¹Dow TL, ²Cross DL, ³Brown JL

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Topic: Reproduction

Presentation type: Oral

Abstract:

Perturbations in serum prolactin secretion, both over and under production, are observed in zoo African elephants that exhibit abnormal ovarian cycles. Similar prolactin problems are associated with infertility in other species. Pituitary prolactin is held under constant inhibition by a hypothalamic-derived neurotransmitter, dopamine; thus, regulation via exogenous treatment with agonists or antagonists may be capable of reinitiating normal ovarian cycles. This study tested the efficacy of oral administration of Cabergoline (agonist) and Domperidone (antagonist) as possible treatments for hyperprolactinemia or prolactin deficiency, respectively. Changes in serum prolactin and progesterone concentrations were evaluated for a year prior and throughout a 12-month treatment period. Hyperprolactinemic (overall mean >30 ng/mL), acyclic female elephants were administered oral Cabergoline (2 mg, n=4) or placebo (dextrose capsule) twice weekly. Overall mean prolactin concentration (heterologous ¹²⁵I double-antibody RIA) decreased in treated females compared to controls (32.22 ± 14.75 ng/mL vs. 77.53 ± 0.96 ng/mL; P = 0.01). Interestingly, overall mean progesterone concentrations (solid-phase ¹²⁵I progesterone RIA) increased slightly (P < 0.05) in treated females (0.15 ± 0.01 ng/mL) compared to controls (0.07 ± 0.01 ng/mL), but no reinitiation of normal cyclic patterns were observed. Prolactin deficient (overall mean <10 ng/mL), acyclic females were orally administered Domperidone (2 g/day, n=4) or vehicle (2 g/day, n=4) for 4 weeks, followed by 8 weeks of no treatment (four cycles repeated in 12-week intervals) to simulate the prolactin pattern observed in normal cycling elephants. Overall mean prolactin concentrations increased (P = 0.005) during treatment (21.77 ± 3.69 ng/mL) compared to controls (5.77 ± 0.46 ng/mL), yet progesterone concentrations were unaltered. In conclusion, prolactin regulation by dopamine was confirmed by expected responses to dopamine agonist and antagonist treatment. However, although prolactin concentrations were successfully altered, with Cabergoline and Domperidone initiating the expected cyclic pattern, neither induced normal ovarian activity in treated elephants.



Abstract #44

Hormonal evidence for reproductive isolation between two species of gray brockets (*Mazama gouazoubira* and *M. nemorivaga*) from Brazil.

¹Martins GS, ²Duarte JMB

¹University of São Paulo, School of Veterinary Medicine and Animal Science, Department of Animal Reproduction/Wildlife; ²São Paulo State University, Faculty of Agricultural and Veterinary Sciences, Deer Research and Conservation Center

Presenting and contact person: Martins GS, gabi_smartins@usp.br

Topic: Reproduction

Presentation type: Poster

Abstract:

Reproductive isolation between species with similar morphological features and distribution is generally confirmed through the reproductive evaluation of hybrids. To assess reproductive isolation between the Brazilian gray brockets (*Mazama gouazoubira* and *M. nemorivaga*) we conveyed intra and inter-specific crosses that resulted in two male hybrids and three pure male offsprings. The poor breeding success observed were mainly due to the scarce number of *M. nemorivaga* specimens and decreased male libido. Fecal samples were collected once a week, from six to 20 months of age. Fecal metabolites of testosterone (FMT) concentrations were determined by enzyme immunoassay (R156/7 polyclonal antibody; C. Munro, UC Davis, USA) previously validated using the process described by Brown et al (2004). Semen analysis and histological testicular examination were performed at 20 months of age. The two hybrids (namely H1 and H2) were products of different crosses and, therefore, were analyzed separately.

Mean and maximum FMT values overlapped within pure offspring, with *M. gouazoubira* values of 1123.75 ± 304.46 ng/g dry weight (DW) and 1486.56 ng/g DW and *M. nemorivaga* values of 881.10 ± 314.082 ng/g DW and 1504.80 ng/g DW. Sperm motility and concentration values were lower for *M. gouazoubira* (52% and 1955×10^6 /ml) than for *M. nemorivaga* (70% and 2250×10^6 /ml). Both species presented normal testicular structure. However, H1 presented the lowest FMT values (747.77 ± 172.98 ng/g DW and 957.90 ng/g DW), azoospermia and testicular hypoplasia. H2 presented FMT values similar to pure offspring (815.82 ± 269.01 ng/g DW and 1163.86 ng/g DW), but oligospermia (motility below 1% and sperm concentration of 2×10^6 /ml) and partially hypoplastic testis.

These results indicate a possible reproductive failure of hybrids by the study of hormone analysis, confirmed by seminal and histological data. Yet, a much larger sample is needed to conclude the existence of reproductive isolation between both species.



Abstract #46

Noninvasive endocrine monitoring of reproduction based on breeding records in Indian mouse deer (*Moschiola indica*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

Mouse deer or Indian chevrotain (*Moschiola indica*) plays a major role in forest ecosystem as a seed disperser and its an important prey for small and large carnivores; it is threatened due to extensive poaching in India. Despite its importance, information on breeding and the reproductive physiology of this species is limited in the wild and captivity. As part of conservation breeding programme, we recorded behavioral observations and discovered unique postpartum estrus that depends on the involution of uterus and the onset of a postpartum ovarian activity in the female reproductive system after parturition. The present study aimed to standardize Enzyme immunoassays (EIAs) for monitoring the reproductive biology of female mouse deer using progesterone (Monoclonal antibody, CL425) and estradiol (Polyclonal antibody, R0008) metabolites. High pressure liquid chromatography (HPLC) analysis revealed the presence of immunoreactive progesterone and estradiol metabolites in feces. A total of 700 fecal samples were collected from 11 adult captive female mouse deer over a period of one year from Nehru Zoological Park, Hyderabad, India. Post-partum estrus was observed after parturition, as evidenced by estrogen peaks during 2-5 days before and after delivery and subsequently returned to the baseline. The mean (\pm SEM) concentration of faecal estrogen was 298.5 ± 76.6 ng/g, 2-4 days before and 228 ± 30.7 ng/g, 2-5 days after parturition. The mean (\pm SEM) concentration of faecal progesterone metabolites were significantly elevated in pregnant (41254.7 ± 1497 ng/g) compared to non-pregnant animals (11277 ± 745 ng/g) ($P < 0.001$). Mounting, mating and postpartum estrus were observed immediately after delivery of fawn (within 6 hrs. of parturition) and recorded as unusual behaviors in this deer. Faecal progesterone and estrogen EIAs were a useful and reliable tool for non-invasive endocrine monitoring and pregnancy detection in captive Indian mouse deer.



Abstract #47

SERUM AND FAECAL CORTICOIDS IN TAPIRS (*Tapirus terrestris*)

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Topic: Stress

Presentation type: Poster

Abstract:

To assist the conservation of *Tapirus terrestris* (Brazilian tapir), this study performed an experimental assessment of serum and fecal corticoids by radioimmunoassay (RIA) because since chronic stress can affect homeostasis and spoil reproduction in wild and captive animals. Blood samples from the cephalic vein and feces directly from the rectal bulb were collected from seven Brazilian male tapirs kept in captivity at the Itaipu Biological Refuge (Brazil) and Itaipu Zoo (Paraguay), from November 10th to 14th, 2014, during the first phase of the project: Andrological parameters and cryopreservation of tapir semen. Plasma was obtained from the blood samples and analyzed by radioimmunoassay (RIA) with the Corticosterone I125 RIA double ImmChemntibody kits (MP BIOMEDICALS, LLC, Orangeburg, NY-USA) and for serum measurements, in addition cortisol measurements was also mensured performed using a commercial diagnostic set (MP BIOMEDICALS, LLC, Orangeburg, NY-USA). Hormone analyses concentrations protocol followed the manufacturer's instructions recommendations, described on the kits label. Fecal samples were lyophilized in a rotary evaporator to eliminate variability of water content in feces and to avoid bacterial contamination. Processing was conducted realized by the Laboratory of Hormonal Dosage of the University of Sao Paulo. The following results were obtained for corticosterone and serum cortisol, respectively: arithmetic mean= 2.8 ng/ml (range of 0.75–5.02 ng/ml) and 3.4ng/ml (range of 0.1–6 ng/ml) arithmetic mean = 4.32 ng/ml. The results obtained from fecal extraction in dry feces were: arithmetic mean = 173.36 ng/g (range of 93.75–265.81 ng/g). For quality control, the intra-assay coefficient of range obtained were corticosterone 1.22% and cortisol 3.0%. This is an experimental study and literature regarding the measurements of corticosteroid hormones in tapirs is scarce therefore there is not enough information to discuss the results obtained in the present study. Also, further monitoring of the animals and evaluation of stress factors during samples collection would improve our analysis.



Abstract #48

ANDROGENS IN TAPIRS (*Tapirus terrestris*)

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Topic: Reproduction

Presentation type: Poster

Abstract:

The conservation of the lowland tapir (*Tapirus terrestris*) is very important for the Brazilian fauna due to its function within the ecological chain. The understanding of their reproductive physiology can guarantee a better management of the captive and wild individuals of this species. The objective of the present study was to perform a comparative study between testosterone levels in serum and feces of *Tapirus terrestris*, but there is still no validation. Samples used in the experiment were collected simultaneously from November 10 to 14, 2014, from seven captive healthy males, all in reproductive age at ITAIPU Binacional (Brazil and Paraguay). Samples were processed at the Laboratory of Hormonal Dosages (LDH) of the University of Sao Paulo (USP). Before the extraction of the androgens, fecal samples were lyophilized to remove the influence of the amount of water in each sample. Fecal testosterone metabolites were extracted according to Graham et. al. (2001), with slight modifications (methanol 90%). The chosen method for hormonal analysis was radioimmunoassay (RIA), with direct testosterone (Immunotech Beckman Coulter, Prague, Czech Republic) accordingly to the manufacturer's instructions. The quality control intra-assay coefficient of variation (CV) was 0.62%. The following results (expressed as mean \pm standard deviation) were obtained: serum testosterone = 0.47 ± 0.61 ng/ml (range 0.01 – 1.79 ng/ml); testosterone metabolites in fecal samples = 5.04 ± 7.07 ng/g (range 0.01 – 15.99 ng/g). The coefficient of correlation between serum and fecal testosterone results was -0.21, but these samples were collected in the same anesthetic procedure. Considering that the transit time for particles in lowland tapir is 24 ± 12 h according to Clauss et. al. (2010), testosterone fecal metabolites represent the day before serum testosterone levels. A comparative serum and fecal reference will be proposed for future evaluation on tapir reproduction.



Abstract #52

The alternative application of urinary specific gravity for the normalisation of endocrine metabolite concentrations in giant panda (*Ailuropoda melanoleuca*) reproductive monitoring

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Topic: Methodologies

Presentation type: Oral

Abstract:

Reproductive monitoring for assisted captive breeding in giant pandas is based on behavioural observation and urinary or faecal hormone analyses. In urinary samples, interpretation of results is compromised by the need for normalisation due to an animal's changing hydration status. Correction of urinary metabolite concentrations based on the creatinine content is universally accepted as the gold standard.

In this study, a largely unexplored, easy-to-perform technique for normalisation, based on urinary specific gravity (USpG) (Pal-USG (Cat), AtagoTM), was examined and compared to creatinine (DetectX®, Arbor AssaysTM). Therefore, a successful reproductive cycle of a 7-year old female panda was monitored through urine analysis with commercial enzyme immunoassays (DetectX®, Arbor AssaysTM), respectively designed for oestrogen, progesterone, ceruloplasmin and 13,14-dihydro-15-keto-PGF2a (PGFM) detection, with each urinary metabolite concentration corrected based on both USpG and creatinine. The investigated urine samples were daily aspirated from the ground from pro-oestrus (February 2016) until parturition (June 2016).

The Pearson's correlation between the creatinine concentration and USpG was high ($r = 0.894$; $p < 0.001$), however, remarkably lower during primary progesterone rise ($r = 0.788$, $p < 0.01$). During this period, low (and decreasing) creatinine values (range: 0.01-0.82 mg/mL) were running in parallel with increasing faecal output (range: 3.70-22.00 kg).

USpG-corrected concentrations were generally lower compared to creatinine-corrected values (Wilcoxon Signed Rank test, $p < 0.001$) with frequent outliers in the creatinine dataset, particularly during primary progesterone rise (i.e. maximum progesterone concentration of 999.76 ng/mg (range: 3.54-999.76 ng/mg) versus 37.54 ng/mL (range: 5.69-37.54 ng/mL) for creatinine and USpG correction, respectively).

Consequently, USpG-corrected data rendered smoother profiles, which were less subject to variation and allowed a more straightforward discrimination between primary and secondary progesterone rise, therefore being potentially advantageous for future studies aiming to unravel key giant panda reproductive events, such as (delayed) implantation.



Abstract #53

Glucocorticoid stress response in tigers with reference to tourism in Bandhavgarh Tiger Reserve, India.

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Topic: Stress

Presentation type: Oral

Abstract:

Tiger (*Panthera tigris*) is a critically endangered species, currently occurring in only 0.5% of its historical range with 3000-3500 individuals. On a global scale, tiger populations continue to be unsafe from poaching, habitat loss, prey depletion and excessive anthropogenic disturbances in their habitats. The present study examines the impact of anthropogenic activities in tiger populations in one of the central Indian tiger reserves using faecal glucocorticoid assessment. A total of 181 scats samples were collected from Bandhavgarh Tiger Reserve during tourism (January – March) and non-tourism (August-September) periods in 2015. These samples were genotyped to ascertain species identification using mcb, TIG 678, TIF and NADPH markers and sexed using ZnF-F and ZnR R markers. Data on anthropogenic disturbance including tourism activities, the number of vehicles and visitors were collected directly and also from records of Forest Department. Of the 181 samples collected, 139 were found to be tiger scats (78 females, 61 males). Fecal glucocorticoids were extracted using Brown et al (1994) method and expressed in dry weight. Fecal hormones were analysed by EIA using Cortisol-R4866 antibody (Munro, UC Davis, USA) and as previously reported (Kumar et al 2014). Both males and females glucocorticoid metabolites concentrations were significantly higher in tourism period (January-March), 39.4 ± 3.9 ng/g, than non-tourism period (August-September), 23.34 ± 3.4 ng/g (M-W test $P < 0.05$). Overall, the females were found to be more stressed than males during both periods. Within tourist season, tigers found in visitor areas had significantly higher glucocorticoid concentrations, 54.32 ± 2.9 ng/g than in other areas, 24.45 ± 3.9 ng/g (M-W test $P < 0.001$). We also found a significant correlation between the number of tourist vehicles and glucocorticoid concentrations ($r = 0.79$; $P < 0.05$). This study once again proves that unsustainable tourism and other anthropogenic disturbance have a direct impact on the physiology of tigers.



Abstract #56

Physiological stress response in *Tamandua tetradactyla* to anthropogenic disturbances: management recommendations to preserve animal welfare in zoos

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Topic: Stress

Presentation type: Lightning Talks

Abstract:

Zoo-housed animals are frequently exposed to environmental challenges due to management routines. These are applied on a daily basis (such as feeding and cleaning) or less frequently (such as veterinary examinations and rotations between enclosures), triggering different stress responses. Thus, evaluating these responses is useful to improve management strategies. We assessed *Tamandua tetradactyla* adrenocortical response to anthropogenic disturbances due to management in Córdoba Zoo. Adult animals (3 male and 3 female) were individually housed during autumn. Animals were exposed to the following procedures: weight (day 3) and health status (blood collection, body temperature and biometrics; day 18) checks (10 minutes per procedure), and to rotations (days 10 and 24) between enclosures. Fresh feces were collected daily over a 34 day-period. Fecal glucocorticoid metabolites (FGM; ug/g wet feces) were extracted using methanol (80%) and measured by an 11-oxoetiocholanolone enzyme immunoassay. Statistical analysis revealed sex (males: 3.7 ± 0.3 > females: 2.5 ± 0.2 ; $P < 0.05$) and individual differences ($P < 0.0001$). Procedure effects (3 post-procedure days) were assessed by calculating the percentage of change from baseline (pre-procedure) in concentrations of FGM. Weight check did not affect FGM concentrations, but health status check increased them (91% change; $P = 0.04$). First rotation had no effect on FGM concentrations; remarkably, the second rotation increased them (178 % change; $P = 0.04$). Based on our results, sex and identity must be taken into account in order to make an adequate use of FGM measurements as a welfare indicator. Animals responded differently to veterinary examinations; weight check did not affect adrenocortical activity, but health status check was stressful. Although rotations between enclosures may be desirable to offer environmental opportunities for exploring, this practice would increase adrenocortical activity when preceded by a stressful situation. Finally, avoiding continuous unpredictable challenges due to management is recommended to prevent compromising animal welfare.



Abstract #59

Gender Differences and Challenges with Determining Seasonal Testosterone Concentrations in Male and Female Sand Tiger Sharks (*Carcharias taurus*)

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Topic: Aquatics

Presentation type: Oral

Abstract:

Sand tiger sharks (*Carcharias taurus*) are a charismatic species that are a great addition for any aquarium collection; however, their dwindling numbers in the wild make ex situ breeding a crucial next step in the management of this species. Hormone monitoring is a useful tool to help breeding populations of animals under managed care. Testosterone has been shown to be a useful indicator of reproductive events in male and female elasmobranchs; A testosterone (R156/7) EIA was validated for male and female sand tiger sharks. Serial dilutions of male and female plasma demonstrated parallelism to the standard curve and recovery of known amounts of testosterone yielded 96% ($r^2=0.998$), in females but 18% ($r^2=0.998$) for males. Male plasma required pretreatment with dissociation reagent (Arbor Assays, cat# X017) for appropriate recovery of known testosterone (105%, $r^2=0.999$). However, similar treatment of female samples resulted in overestimated hormone concentrations (150%, $r^2=0.996$), but diluting the reagent 1:4 prior to sample treatment achieved recoveries of 104%, $r^2=0.999$. Attempts to reduce the dissociation reagent for males resulted in reduced recovery (67%, $r^2=0.997$) so 100% strength was used in males and 25% strength in females to attempt to standardize treatment. Males ($n=32$) had lower ($P<0.05$) testosterone in summer months (Jun-Aug; 35.6 ± 3.5 ng/ml) compared to winter (Dec-Feb; 64.4 ± 7.5 ng/ml), spring (Mar-May; 66.1 ± 7.7 ng/ml) and fall (Sep-Nov; 61.5 ± 6.4 ng/ml), whereas females ($n=13$) had lower ($P<0.05$) testosterone in fall (7.3 ± 0.3 ng/ml) compared to winter (9.6 ± 1.7 ng/ml), spring (14.2 ± 2.6 ng/ml) or summer (14.3 ± 2.3 ng/ml). Next steps include further investigation into seasonal testosterone changes in sand tiger sharks and continued sampling to document bi-annual endocrine cycles in females.



Abstract #60

Validation of non-invasive fecal reproductive hormone assays for the northern fur seal (*Callorhinus ursinus*)

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Topic: Reproduction

Presentation type: Poster

Abstract:

The northern fur seal (NFS; *Callorhinus ursinus*) is a vulnerable species that has experienced drastic, unexplained population declines in recent years. It is, therefore, increasingly important that aquarium populations of NFS are stable and breeding optimally. Restricted numbers of aquarium-housed NFS, combined with limited available tools to assess reproductive events including pregnancy and puberty onset, has hindered breeding success. Previous endocrine studies on NFS used serum hormones, however, longitudinal fecal hormone measurements may provide new insights into reproductive patterns. This pilot study was performed to validate fecal assays and compare concentrations of three reproductive hormone types (progestagens, androgens, estrogens) in NFS. Samples, collected from seven NFS (4 females, 3 males) housed at New England Aquarium and Mystic Aquarium, were freeze-dried and 0.2 g feces was extracted with 2.0 ml 90% methanol. Parallelism and accuracy validation tests were conducted on pooled extracts, with an enzyme immunoassay (EIAs: Arbor Assays DetectX progesterone (#K025), testosterone (#K032), estrone-3-glucuronide (E1G; #K036)) and radioimmunoassay (RIAs: in-house progesterone (Munro CL425), in-house testosterone (Niswender #250), MP Biomedicals total estrogens (#07-140202)) tested for each hormone type. RIAs failed parallelism tests, whereas EIAs demonstrated strong parallelism ($P > 0.05$). EIAs also passed accuracy checks, with slopes not significantly different from 1 ($P > 0.05$; slope progesterone: 0.92, testosterone: 1.2, E1G: 0.97). Hormone measurements were then performed on individual fecal samples ($n=59$). Initial comparisons across sexes and age classes showed females had significantly greater progestagen concentrations compared to males (mean females: 3,801.2 ng/g, males: 224.8 ng/g; $F=196.09$; $P < 0.001$); and all subadult groups had lower E1G concentrations than adults (subadults: 9.1 ng/g, adults: 10.9 ng/g; $F=6.52$; $P < 0.05$). Within males, testosterone was greater in adults compared to subadults (adults: 257.9 ng/g; subadults: 188.7 ng/g; $t=2.18$; $P < 0.05$). Non-invasive endocrine monitoring will enhance collection care and management, and has applications for studying reproductive failure in wild NFS populations.



Abstract #62

Using serum biomarkers to assess the health of African and Asian elephants

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Topic: Animal health and welfare

Presentation type: Poster

Abstract:

Elephants experience a range of health issues that can impact their well-being and survival, including infectious diseases, degenerative conditions and gastro-intestinal issues. Several of these conditions lack early diagnosis tools, effective treatments and sometimes even the underlying immune response is not thoroughly understood. The measurement of serum biomarkers indicative of inflammation have proven beneficial to health assessments of both laboratory and domesticated species, and provide an exciting avenue for monitoring, diagnosing and treating health problems in wildlife species, like elephants. Acute phase proteins (APPs) and cytokines involved in the immune response to inflammation, infection, stress and trauma, all play a role in promoting healing and restoring homeostasis. Pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin (IL)-6 and IL-1beta play a role in mediating the inflammatory response, interferon-gamma (IFN-g) is involved in the induction of cell-mediated immunity, and anti-inflammatory cytokines such as IL-10 act to promote healing. Together these markers could prove beneficial to understanding the elephant immune response to a range of pathologies, and be used to aid diagnosis and assess treatment efficacy and prognosis. Serum samples were collected from 125 African and 104 Asian elephants across North America, from both healthy individuals and those exhibiting signs of illness and injury. These were analyzed for seven different serum markers (APPs serum amyloid A (Eiken) and haptoglobin (Tridelta); cytokines TNF- α (Thermo Fisher), IFN-g, IL-1beta, IL-6 and IL-10 (all R&D Systems)), and normal reference ranges and clinical values established. Many of these markers were undetectable in clinically healthy individuals, but increased by several orders of magnitude during illness and injury, ranging from 50 to more than 2500-fold higher than baseline. This information will focus future research priorities, and help with the management of elephant morbidity and mortality, both under human care and in the wild.



Abstract #63

It's not all black and white: urinary oestrogens as an implantation marker in the Giant Panda (*Ailuropoda melanoleuca*)

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Topic: Reproduction

Presentation type: Lightning Talks

Abstract:

Urinary hormone monitoring in giant pandas is carried out regularly to determine the female reproductive state. This practice is well established in determining estrus by measuring estrogens. Monitoring a potential pregnancy is complicated by embryonic diapause and pseudopregnancy. The profile of estrogen is currently undefined during diapause and gestation, and is suggested to remain at baseline throughout (pre-estrus levels). Our objective was to examine estrogen concentrations during the luteal phase and around putative implantation.

Urine was collected from three female pandas over five reproductive cycles representing two births, two unsuccessful pregnancies and one non-bred cycle. Estrogen concentrations were measured by DetectX® Arbor enzyme immunoassays (Estrone-3-glucuronide or Estrone-3-sulphate), and corrected against creatinine (Cr) concentration.

Estrogen concentrations fluctuated during the primary progesterone rise (0.5-15 ng/mg Cr). In bred cycles, estrogen concentrations increased from 95±21 days post-AI for 12.5±0.5 days upon entry into the secondary progesterone rise (fold change 3.15 [95% C.I. 1.16-5.14]). All cycle estrogen concentrations were significantly higher prior to reaching peak progesterone ($P < 0.05$, AUC). Estrogen concentrations decreased to sub-baseline levels for the remainder of the luteal phase (0.3-8 ng/mg Cr; 30.5±10.5 days). The successful pregnancies displayed an identical estrogen pattern at 37 days-to-birth, followed by sub-baseline concentrations from 23 days until birth. The profile at 37 days-to-birth was replicated in the unsuccessful cycles, but not the non-bred cycle.

In conclusion, estrogens did not remain baseline in the monitored reproductive cycles, indicating a potential role in diapause and gestation. We propose that 37 days-to-birth has the potential to be identified as the timing of implantation in these pandas. Although all bred estrogen profiles were similar, with further analysis the unsuccessful cycles were deemed post-implantation losses. Further investigation is required, however this study suggests estrogens have a potential role in successful embryo implantation in the giant panda.



Abstract #65

Validation of a fecal glucocorticoid metabolite (FGM) assay and field extraction methods to monitor welfare of mandrills (*Mandrillus sphinx*) reintroduced into Republic of Congo (RoC)

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Topic: Methodologies

Presentation type: Poster

Abstract:

In 2013-2015, the Jane Goodall Institute released confiscated wild-born mandrills into RoC. We monitored FGMs in these individuals, as stress is implicated as a major factor in animal reintroduction failure. Our objective was to select a suitable EIA to quantify stress in mandrills and validate field extraction methods.

We evaluated a panel of assays in zoo-managed mandrills before/after medical procedures as a biological validation, and separated fecal mandrill extracts into fractions using HPLC to compare fraction immunoreactivity with elution times of hormone standards. We assessed the effects of various extraction methods (7 treatments:e.g., variable extraction times) on [FGM] using a pooled mandrill fecal sample (n=10 subsamples/treatment) and used GLM or ANOVA/post-hoc Dunnett's T-tests where appropriate.

We selected the 11- β -hydroxyaetiocholanolone assay (69a; R. Palme) to monitor mandrills based on concentrations and relative increases following medical procedures. Slopes were not significantly different between pooled mandrill samples and standard curve ($P=0.84$). Exogenous 69a added to mandrill fecal extracts yielded $98\pm 1.5\%$ recovery. Male and female fecal extract HPLC fractions had minimal immunoreactivity in cortisol, corticosterone, and testosterone EIAs. Highest 69a immunoreactivities for the male extract were at retention times for an unknown compound and the 69a standard. The female extracts had highest 69a immunoreactivity where 69a eluted.

We found a significant effect of sample treatment on [69a] ($P<0.001$). Samples immediately extracted and frozen resulted in ~2-4x higher [69a] than samples with delayed extraction ($P<0.05$) or drying times ($P<0.05$). Lowest extraction yield was when samples rested at RT 8h before ethanol addition ($P<0.05$). Samples in ethanol for longer (>48h) had higher [69a] than those held for shorter intervals (<24h; $P<0.05$).

We validated the use of the 69a EIA for monitoring mandrill welfare. Employing consistent time before extraction and extraction time is important for accurate comparisons among samples/individuals/sexes in this species.



Abstract #67

Effect of fecal sample moisture content on extraction efficiency and progesterone concentration in Red River Hog
(*Potamochoerus porcus*)

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Topic: Methodologies

Presentation type: Oral

Abstract:

Fecal steroid metabolite extraction is a non-invasive technique utilized by zoo and wildlife scientists to monitor endocrine function. The gold standard extraction technique utilizes dried feces and a radioactive tracer to monitor extraction efficiency. The availability of drying equipment and radioactive tracer for the measurement of extraction efficiency is limited in a zoological or field setting. This study compared the effect of moisture removal via lyophilization on extraction efficiency and progesterone concentration in fecal samples from captive, female Red River Hog (*Potamochoerus porcus*). Fecal samples were collected three times per week for 4-5 months from female Red River Hog in breeding pairs (n=2). Samples (0.2 g) to which 1000 cpm of ³H-progesterone (Perkin Elmer, Waltham, MA, USA) was added were double extracted with 90% ethanol by boiling, followed by centrifugation, and collection and evaporation of the ethanol supernatant under compressed air. Evaporated extracts were resuspended in 100 ul of pure ethanol and 900 ul of assay buffer. Extraction efficiency was assessed by addition of 100 ul of extraction in scintillation fluid (Ultima Gold, Perkin Elmer), which was counted using a unique quench curve generated for each animal and moisture condition. Extracts were diluted 1:1000 and run on a progesterone ELISA using the CL425 monoclonal antibody diluted 1:300,000 and HRP diluted 1:20,000 (Coralie Munroe). Data was assessed for normality by univariate analysis and logarithmically transformed as needed followed by analysis with a Wilcoxon matched pairs sign ranked test (SAS, Inc, Cary, NC, USA). The extraction efficiency (Dry: $> 100 \pm 3.0\%$; Wet: $98.4 \pm 3.0\%$; $p < 0.0001$) and progesterone concentration (Dry: 666.5 ± 65.0 ng/g feces; Wet: 346.1 ± 65.1 ng/g feces; $p < 0.0001$) were significantly greater for dried samples. In conclusion, removal of sample moisture may yield a more accurate estimate of actual fecal steroid hormone concentrations in this species.



Abstract #68

Androgen correlates of throat colouration in the Southern Ground-Hornbill (*Bucorvus leadbeateri*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

The Southern Ground-Hornbill (*Bucorvus leadbeateri*; SGH) is a slow breeding and group-living (2-12 birds) species, with groups comprising a single breeding alpha pair, consisting of a red-throated male and a blue-throated female. The remaining group members do not breed, and females are evicted from their natal group once their sexually dichromatic, blue throat colouration begins to show. However, recent discoveries suggest that several birds originally assigned as females were misidentified, as they are actually males displaying a female throat colouration.

To better understand the factors influencing throat coloration in SGH, we examined the suitability of an enzyme-immunoassay to monitor androgen metabolite concentrations (fAM) in SGH droppings. A total of 69 birds (1-8 droppings per bird) of various ages, demographics, and origin were sampled and steroid extracts (n=282; 0.05-0.06g dry faecal matter extracted with 1.5ml 80%EtOH) measured for fAM concentrations using an antibody against testosterone-3-CMO:BSA and 5 α -androstane-3 β ,17 β -diol-3-HS:DADOO-biotin as label. The assay was biologically validated for SGH by demonstrating significantly higher fAM concentrations for adult males compared to subadult/juvenile male birds (T26,14 = 175; p = 0.002). Of the 26 adult males monitored, 6 animals showed red throat colouration (R-birds), 10 birds had small blue patches (sB-birds), and 10 males showed distinct blue areas on the throat (B-birds). Although not statistically significant different (H2 = 3.69; p = 0.16), overall individual median fAM concentrations of B-birds were 90% (sB-birds 38%) higher compared to respective hormone levels for R-birds. Additionally, adult blue throated females showed significantly higher fAM concentrations compared to subadult/juvenile female birds (T25,13 = 353; p = 0.002).

The role of androgens in terms of throat colouration needs further investigation in order to better understand the role of blue males in the social system of SGH for optimizing future management practices in captive breeding and reintroduction programmes.



Abstract #69

Comparative evaluation of gestation in three rhinoceros species (*Diceros bicornis*; *Ceratotherium simum*; *Rhinoceros unicornis*)

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Topic: Reproduction

Presentation type: Lightning Talks

Abstract:

Monitoring progesterone metabolite concentrations is routinely used for diagnosing pregnancy in all species of rhinoceroses kept in captivity. The length of pregnancy ranges from 15 to 16 months. For a precise analysis of gestation data results from almost 20 y of monitoring were comparatively analyzed between the Black Rhinoceros (*Diceros bicornis*), the White Rhinoceros (*Ceratotherium simum*), and the Indian or Greater One-horned Rhinoceros (*Rhinoceros unicornis*). Mean \pm SEM values of gestation lengths for the three species were 463.0 ± 1.71 d; 500.5 ± 1.86 d, and 483.3 ± 2.23 d, respectively. Gestation length varied by 3 – 4 weeks in all three species; confirmed gestation lengths ranged between 452 – 475 d; 490 – 510 d, and 462 – 497 d in the Black, the White and the Indian rhinoceros. Fecal progesterone metabolites were analyzed using established group-specific enzyme immunoassays for 20-oxo-pregnanes in the Black and the White rhinoceros, and 20 α -OH-pregnanes in the Indian rhinoceros. Mean fecal pregnane values from N = 26, 34 and 23 pregnancies for the three species were calculated and revealed considerable differences in the onset of placental steroid production. Mean 20-oxo-pregnane concentrations continuously increased between days 75 – 125 in the Black, and between days 65 – 150 in the White rhinoceros; 20 α -OH-pregnane values in the Indian rhinoceros increased between days 100 – 175. Remarkably, a rather wide range in the onset of placental pregnane production between days 55 – 150 was observed in individual Indian rhinoceroses. In conclusion, long term endocrine monitoring of gestation revealed a high degree of variability in gestation length and endocrine physiology of the three rhinoceros species studied.



Abstract #70

Volatile and non-volatile bioindicators related to reproductive status in giant panda urine (*Ailuropoda melanoleuca*)

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Topic: Methodologies

Presentation type: Oral

Abstract:

As solitary living individuals, giant pandas (*Ailuropoda melanoleuca*) use olfactory cues (i.e., in urine) to mediate mating-related social interactions. In ex-situ panda breeding, urinary steroids serve as estrous indicators and to predict the day of AI. Following that, female pandas generally experience pseudopregnancy. However, if a true pregnancy occurs, a hormone producing placenta will be formed that may affect the composition of urinary steroids. Additional reproductive status related indicators could be polyamines. In rodents, the production of polyamines has been shown to be altered during pregnancy.

We aimed to: 1) analyse urinary volatiles that adsorb on polydimethyl-siloxane (PDMS); 2) compare the steroid profiles between pregnant and pseudopregnant pandas; and 3) investigate whether urinary polyamines are related to reproductive status.

An Agilent gas chromatography mass spectrometer (GCMS) was used for the analyses of PDMS adsorbed substances, urinary steroids (after hydrolysis, extraction and derivatization), and urinary polyamines (after derivatization) using a single 1 ml portion of urine.

Analyses of PDMS adsorbed urinary volatiles revealed an abrupt increase of fatty acids from basal to peak levels 6 days prior to ovulation that had been used for an early ovulation prognosis in one panda. However, comparing fatty acid profiles between pandas over consecutive years revealed differences in fatty acid composition between individuals and between years.

When profiling urinary steroid metabolites, the expected peak in estrone-sulfate was confirmed on the day prior to ovulation. The major gestagen metabolites found in panda urine were 5a- and 5b-pregnane-3a-ol-20-one and pregnanediol (5b-pregnane-3a,20a-diol). Both metabolites are products of deviating metabolic routes. No difference in progestagen composition was found between pregnancy and pseudopregnancy in the same panda.

Comparing the polyamine composition so far has not revealed a clear picture useful for distinguishing pregnant and pseudopregnant giant pandas. This might be related to nutritional effects that obscure polyamine profile differences.



Abstract #71

Higher levels of cortisol in-utero in nutria even-sex-ratio litters

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Topic: Reproduction

Presentation type: Oral

Abstract:

Precocious mammals grow hair as early as the second trimester. Quantifying hair steroids from fetuses allows a glimpse of conditions in utero. In this study, we used a wildlife invasive species model, the nutria (*Myocastor coypus*), to examine the interactions between litter sex ratios, individual fetuses' intrauterine position, and long-term integrated cortisol levels. We used animals taken from the wild and determined fetal age using an established formula adapted for its use in nutria. Hair cortisol was quantified using a commercial ELISA kit (Salimetrics Europe, Newmarket, UK). Using hair-testing in 22 litters (121 fetuses), we found that litter sex ratio explained 51% of the variation in litters average cortisol levels. Litters with even sex ratios tended to have the highest mean cortisol levels ($R^2=0.51$; $n=22$; $P=0.001$). The same was found in individual fetuses (model $R^2=0.61$; $F_{1,25}=12.22$, $P=0.0018$). In addition, litters with even sex ratio are characterized by a highest number of fetuses adjacent to an opposite-sex neighbor. We found that fetuses that were next to the opposite sex in utero had longer trunk (length from shoulders to the base of tail) than those next to neighbors of the same sex (model $R^2=0.89$; $F_{1,112}=8.47$, $P=0.0044$). Studies show that cortisol has a crucial role in lung and surfactant system maturation in utero, preparing the fetus for extra-uterine environment. These findings suggest a possible advantage to heterogeneous sex ratios, and open exciting opportunities to learn about fetal conditions and the uteral environment.



Abstract #72

Are we overestimating the utility of hair glucocorticoids? Early findings from a systematic review in progress

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Topic: Stress

Presentation type: Oral

Abstract:

The fastest growing method for quantifying stress using glucocorticoids is determining their concentrations in hairs. Whether collecting hair-snag samples in the wild, shaving hairs from captive animals, or cutting locks from human patients, the method has found a number of uses. The growing popularity of the method is contrasted with conflicting evidence and differing interpretations of the measurements in relation to physiological and psychological stress: What, exactly, do hair glucocorticoids tell us of a subject?

In 2016 we set out to compile a comprehensive systematic review of all the published literature on hair glucocorticoids published before January 2016; from wildlife, to captive animals, to human studies. The goal of the study was to critically assess the empirical evidence that hair glucocorticoids are a long-term marker of stress.

Having screened 472 publications, we found that many of the studies had been conducted in uncontrolled settings, with little to no precautions made for eliminating sources of bias. A clear majority of the investigated studies were of an exploratory nature, with no pre-specified hypothesis and no possibility for substantiating the accuracy and veracity of the findings. Focusing instead on the controlled studies (n = 64), we found that the method appears to detect stress under certain conditions, but that there were a number of unresolved issues. Of particular note was the complete lack of evidence supporting hair glucocorticoids as a marker summarizing HPA axis functioning over longer time scales (months, as is often claimed).

Going forward we would do well to temper our expectations when using hair glucocorticoids as markers of stress. The method is sure to have its uses, but the quality of the data making up the method's foundation needs to be elevated and we recommend practicing caution when taking published findings at face value.



Abstract #73

Statistics in wildlife endocrinology: A review of current practice and recommendations for future directions

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Topic: Methodologies

Presentation type: Oral

Abstract:

Statistical analysis forms a cornerstone of several scientific disciplines and can be required for publishing in peer-reviewed journals. However, not all research requires statistics for robust inference, and statistics can be misused or abused to generate false inference. We set out to determine how statistics are currently used in the field of wildlife endocrinology, whether these practices are appropriate, and how we can improve our approaches moving forward. To do this, we reviewed articles published in peer-reviewed journals over the last five years (2013 – 2017). *A priori*, we developed a list of criteria by which to assess the quantitative methods used in the studies. We searched for articles using an electronic bibliographic database tool, searching on the terms “wildlife” and “endocrinology” in the topic field. We also assessed existing datasets containing wildlife hormone data to determine the type and statistical distribution of data contained therein. In general, we found that hormone data do not follow a normal distribution, but rather a log-normal distribution. Most researchers do not report the distribution of their data, but often apply frequentist parametric statistics with or without the necessary data transformations. Statistical significance is often reported without mention of an effect size or an interpretation of biological relevance. Current application of statistics to wildlife endocrinology can be improved by taking the following steps: 1) devise *a priori* questions and hypotheses; 2) determine from literature or pilot studies the statistical distribution of hormone data, the possible variance in data, and possible biologically-relevant effect sizes; 3) using this information, perform prospective power analyses, determine required sample sizes, and design appropriate studies (recognising that well-designed studies may not require statistics); 4) conduct and report quantitative analyses with fidelity and without violating key assumptions of the approaches used; 5) provide biologically-relevant context for all results.



Abstract #74

Fecal glucocorticoid metabolite monitoring in kea (*Nestor notabilis*) during Avian Bornavirus outbreaks at the Cincinnati Zoo and Botanical Garden

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

It is well documented that prolonged stress can negatively influence immunity. When kea at the Cincinnati Zoo and Botanical Garden (CZBG) started exhibiting symptoms of avian bornavirus (ABV) that sometimes led to mortality, keepers suspected that stress associated with management or environmental changes could be triggering ABV activation in the CZBG flock. The main objective of this study was to determine if elevated fecal glucocorticoid metabolites (FGM) were associated with ABV symptoms in individual keas. The secondary objective was to ascertain if FGM concentrations were impacted by season in the kea flock. Samples were collected from the individuals for over a year. Collected samples ($n = 255$) were dried and FGM extracted via shaking in 70% ethanol. Extracts were dried and re-constituted with assay buffer. Samples were then analyzed for corticosterone using an antibody developed by Coralie Munroe at UC Davis (CJM006), and the Arbor Assays double antibody competitive enzyme immunoassay method. Great record keeping by the kea keepers gave exact dates of symptom onset for individuals. FGM concentrations in samples collected during the twenty days pre- and post-symptom onset were analyzed. FGM concentrations were higher in post- versus pre-symptom samples (118 pg/g versus 66 pg/g, respectively; $p = .035$). Further data analysis of seasonal impacts on FGM revealed that females have higher ($p = .027$) corticosterone levels during the breeding season than the non-breeding season (107 pg/g and 65 pg/g, respectively). Males do not exhibit this difference (breeding = 57.5 pg/g, non-breeding = 58 pg/g). In conclusion, FGM concentrations were not elevated prior to onset of symptoms indicating that a stressor or excitatory event is not triggering ABV outbreaks in the CZBG kea flock. Moreover, the only seasonal differences observed were in females during the breeding season suggesting excitatory or stressors associated with breeding and not season were responsible.



Abstract #75

A preliminary investigation for using olfactory stimulation as a method for improving cyclicity in captive female African black rhinos

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Topic: Reproduction

Presentation type: Oral

Abstract:

Captive black rhinos (*Diceros bicornis michaeli*) cannot be kept in breeding situations all the time due to unavailable genetically compatible mates, health, or lack of space for new individuals. Long non-reproductive periods have been associated with sub-optimal reproduction in both male and female rhinos of several species, including the black rhino. The objective of our study was to determine if olfactory exposure could stimulate and/or maintain normal estrous cycle dynamics and elicit estrual behavior in captive female black rhinos in a non-breeding situation. Five females housed at three different North American facilities were exposed to two olfactory stimuli: fecal samples from a proven male conspecific and vanilla extract (control) for 8 weeks. Fecal samples were collected every other day for 8-12 weeks prior to olfactory exposure (pre-exposure baseline) and continued for the duration of the exposures treatment. Pre-exposure and exposure baseline gonadal and adrenal metabolite concentrations were calculated using an enzyme immunoassay with a polyclonal antiserum for progesterone (CL425, C. Munro), cortisol (R4866, C. Munro), and corticosterone (CJM006, C. Munro) and validated with a parallelism and recovery check. Chemosensory behaviors were recorded during olfactory exposures using GoPro cameras. Preliminary analyses indicate that in at least one female, baseline progesterone concentrations increased during olfactory exposure (pre-exposure: 80.87 ± 4.27 ng/g; exposure: 173.65 ± 12.38 ng/g) and estrous cycle length began to normalize (pre-exposure: 14.5 ± 3.6 days; exposure: 22.8 ± 6.24 days). Furthermore, conspecific fecal olfactory stimulus elicited a great expression of chemosensory behavior and longer duration of olfactory investigation in comparison to the control. Our results suggest that olfactory cues can potentially be used to maintain or improve reproductive potential during non-breeding periods.



Abstract #76

Evaluating the biological sensitivity of non-invasive methods for measuring adrenocortical activity: an example in cattle

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Topic: Stress

Presentation type: Oral

Abstract:

Faecal cortisol/corticosterone metabolites (FCM) are frequently measured for evaluating hypothalamic–pituitary–adrenal (HPA) axis activity. Unfortunately, utilized methods in roughly one third of all published papers lack a sound physiological validation (demonstrating that an increase in plasma glucocorticoid levels is well reflected in FCM concentrations). Such a validation is frequently performed by injecting a high dose of ACTH. The present study aimed to evaluate in cattle diurnal variations (n=10 cows) in FCM excretion and the biological sensitivity of an 11-oxoaetiocholanolone enzyme immunoassay (EIA) by a dose response experiment over a wide range of intravenously injected synthetic ACTH (Synacthen, CIBA-Geigy, Switzerland) amounts (n=2 each: 0.016; 0.031; 0.063; 0.125; 0.25; 0.5; 1.0 or 3.0 mg per animal). Frequent blood samples (via indwelling catheters) and all voided faeces were collected for 24 hours before (blood only in ten cows) and after the ACTH challenge. Plasma cortisol and faecal cortisol metabolites (11,17-dioxoandrostanes) were measured via EIAs (utilizing antibodies against cortisol-3-CMO:BSA and 11-oxoaetiocholanolone-3-HS-BSA, respectively). Episodic variations (expressed as max. to min. and CV% in individual cows) of FCM were less pronounced than those of plasma cortisol (mean±SD of all cows: 2.4±0.4 vs. 15.4±9.3 times and 26±8% vs. 82±24%, respectively). Even the lowest ACTH dose (0.016 mg) gave a clear signal in both sample types. Dose of ACTH was only well correlated (r=0.819; p<0.0001) with the percent increase (above baseline) of FCM, suggesting that their concentration is a better reflection of HPA axis responses than plasma cortisol values. Less expressed episodic variations in FCM levels warrant them better suited for assessing baseline or chronic HPA activity (especially when only a few samples can be collected). In addition, our group-specific 11-oxoaetiocholanolone EIA (especially designed to pick up a group of faecal cortisol metabolites) is characterized by a high biological sensitivity, which enables the detection of minor stressful events.



Abstract #77

Diagnosis and treatment of persistent corpora lutea in a female Babirusa (*Babirusa babirusa*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

Little is known about reproductive physiology and pathology in non-domestic Suidae. In April 2015, a 10-year-old captive, female Babirusa (*Babirusa babirusa*) presented with history of poor libido and non-fertile matings. She had not displayed estrus since November 2014. Transabdominal ultrasound (US) confirmed she was not pregnant and fecal progesterone (4/2015-6/2015; Arbor Assays, Ann Arbor, MI, USA) were near baseline (range: 200.3-949.6 ng/g feces) with no estrous cycles. The female was not observed to mate November 2014-October 2015. In November 2015, she was anesthetized and transrectal ovarian US revealed large corpora lutea, whereas transabdominal US confirmed she was not pregnant. This information coupled with a history of no mating and a high fecal progesterone concentration just prior to transrectal ovarian US (11/29/2015: 2503.5 ng/g of feces) led to a diagnosis of persistent corpora lutea and treatment with 20 mg prostaglandin (Lutalyse®, Zoetis, Parsippany, NJ, USA) intramuscularly (IM). At the time of prostaglandin administration, the male and female Babirusa were housed together. Intromission was noted 15 days after prostaglandin, when fecal progesterone were at baseline (range: 111.6-151.0 ng/g feces). Despite mating, repeated transabdominal US post-intromission confirmed lack of pregnancy. The female developed persistently elevated fecal progesterone (range: 1421-6420 ng/g feces) again in the absence of pregnancy (3/2016-5/2016). This range is very high compared to the range of fecal progesterone concentrations in cycling Babirusa (~ 100-2400 ng/g feces; Berger et. al. 2006). The female did not mate during 2016, therefore, after verification she was not pregnant via transabdominal US, prostaglandin was administered again in January 2017. Fecal progesterone were at baseline (range: 177.4-330.1 ng/g feces) and mating was noted 20 days post-prostaglandin. This case provides evidence that, despite its rarity in domestic swine, persistent corpora lutea may affect non-domestic Suidae and estrus can be induced in these cases with prostaglandin.



Abstract #78

Sex differences in hypothalamic-pituitary-gonadal axis response in wild gerbils

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Topic: Reproduction

Presentation type: Oral

Abstract:

Gonadotropin releasing hormone (GnRH) plays a major role in the hypothalamic-pituitary-gonadal (HPG) axis, stimulating the secretion of luteinizing hormone from the pituitary gland, which in turn stimulates testosterone release. Circulating testosterone concentrations can differ between sexes, but little is known about sex differences in the HPG axis. We examined sex differences in maximal testosterone secretion in *Gerbillus nanus*, a wild rodent species with no sex difference in circulating testosterone levels. We carried out two studies. In the first, we assigned 40 males and 40 females to a group that received either a beeswax implant with testosterone (4.95, 6.6, or 8.25 mg), or to the control group. Blood was sampled weekly over 7 weeks. In the second study, 20 males and 21 females were either injected with GnRH (50 or 500 ng dissolved in PBS), or PBS only. Blood was drawn before the injection and 60, 90, and 120 min post injection. In both studies, we found significant differences between the sexes. Whereas females increased circulating testosterone concentration with testosterone implants, males did not. On the other hand, while males increased blood testosterone concentrations following GnRH injections, females did not. We suggest that the sexes may have evolved different strategies of HPG axis responsiveness in order to cope with different reproductive and energetic demands.



Abstract #79

Determining the etiology of infertility in male snow leopards (*Uncia uncia*) housed in North American zoos

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Topic: Reproduction

Presentation type: Oral

Abstract:

Little is known about the snow leopard due to its elusiveness in the wild. However, the zoo population can provide an opportunity to study this endangered species and provide a safe haven for its future. Unfortunately, since 2008, <30% of recommended breeding pairs have successfully produced a litter. Because recent research has demonstrated that most females are cycling regularly, our goal was to evaluate male fertility by evaluating spermatozoa production, both fecal glucocorticoid (FGM) and androgen (FAM) metabolite hormone production and environmental conditions. Males (n=11) were electroejaculated once during the breeding season from February through June. Fecal samples were collected from each twice weekly from December through June and analyzed using cortisol (C. Munro antiserum R4866) and testosterone (C. Munro antiserum R156/7) enzyme immunoassays. To evaluate environmental conditions across the 10 facilities, zoo staff answered questions about habitat lighting, diet and social conditions. Results show that mean (\pm SEM) FAM (ng/g dry feces) varied ($P < 0.001$) across males (range, 369.2 ± 33.9 to 992.5 ± 114.5) and peaked ($P < 0.001$) in February (675.6 ± 36.1) and was lowest in June (454.4 ± 17.6). Similarly, mean FGM (ng/g dry feces) varied ($P < 0.001$) across males (range, 55.8 ± 7.9 to 270.4 ± 17.1); however, FGMs were similar ($P > 0.05$) across months. For the semen evaluation, two of 11 males were aspermic upon electroejaculation. The relationship to FAMs is questionable as one male had the highest FAM; however, FAMs peaked in February and semen was evaluated in May. The second aspermic male had one of the lowest mean FAM values that peaked in January and semen was collected in June. Both males had medium to low FGMs compared to other males. Future analyses will investigate the relationship among hormones, semen quality and environmental conditions. These results provide an initial step towards understanding poor breeding success and will assist with improving the sustainability of this AZA population over time.



Abstract #80

Monitoring the behavior and stress physiology of four male gorillas (*Gorilla gorilla gorilla*) during a bachelor group formation

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

Gorillas are polygynous and zoos are challenged with housing males not living in mixed-sex breeding troops. One solution is for zoos to socially manage all-male “bachelor” groups versus solitary housing; however there is little information on how to establish these all-male groups. Therefore, we monitored the physiology and behavior of four male gorillas (6-9 years old; M1-M4) during a bachelor group formation at Lincoln Park Zoo over one year. Our objectives were to monitor fecal glucocorticoid (FGM) and androgen metabolites (FAM), social behaviors (abnormal and agonistic), and proximal inter-individual distance during the bachelor group formation. Data were collected in two 6-month periods: an introduction period (P1) and a subsequent matched period (P2) when all males were housed together. FGM and FAM concentrations were extracted from wet feces with 90% ethanol, then measured by enzyme immunoassay (EIA) using Cortisol-R4866 and Testosterone-R156/7 antibodies (Coralie Munro, UC Davis, USA), respectively. During P1, mean FGMs (ng/g wet feces) were similar ($P>0.05$) across males (M1 28.2 ± 5.5 ; M2 26.0 ± 1.1 ; M3 37.1 ± 2.6 ; M4 57.1 ± 6.1). For P2, FGM remained consistent ($P>0.05$) for M1 and M4, and decreased ($P<0.05$) for M2 and M3. Mean FAMs (ng/g wet feces) were similar ($P>0.05$) across males in P1 (M1 114.7 ± 7.9 ; M2 136.5 ± 15.6 ; M3 114.2 ± 9.8 ; M4 232.0 ± 28.0). For P2, FAM remained consistent ($P>0.05$) for M1, M2 and M3, but increased ($P<0.05$) for M4. Time spent engaging in agonistic (0.13%) and abnormal (1.51%) behaviors was low ($P>0.05$) during P1 and P2. Proximity within dyads was similar ($P>0.05$) between M2-M3 and M1-M3 in P1 and P2, but increased ($P<0.05$) in P2 between M1-M4, M2-M4, M3-M4, and M1-M2. Low levels of stress indicators during P1 and P2 were desired outcomes and indicate a successful bachelor group formation process that can serve as a model for others.



Abstract #81

Discovering the reproductive physiology of Arabian Tahr (*Arabitragus jayakari*) (Thomas, 1894) through non-invasive endocrine monitoring.

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Topic: Reproduction

Presentation type: Oral

Abstract:

The Arabian Tahr is endangered goat-like specie endemic to the Hajar Mountains (1,800mt) that's ranges from UAE to the Sultanate of Oman with an estimated population of 2,000 individuals. In 2005 a captive population was established at MNC for a breeding plan and a total of 23 females were monitorated. Males and females pairs were kept in pens with water ad libitum, and fed with alfalfa hay and Mazuri® Wild Herbivore Diet pellets. In order to assess the ovarian activity of this specie, a non-invasive faecal steroid monitoring was used to evaluate the reproductive events (2005-2007). The enzyme immunoassay (EIA) validation was performed by parallelism, recovery and dose-response (R²: 0.87). The physiological validation was performed by the P4 concentration during pre puberty and non pregnancy periods. The intra-assay and inter-assay coefficient of variation (high and low controls) were kept under 10%. Statistical analyses were performed by R Project using Student's t-Test (p<0.05). In the extraction process, 0.5g of wet feces was added to 5ml of 80% methanol. The supernatant was diluted in EIA buffer (1:200) and all samples were analyzed in duplicate. The concentrations of progesterone metabolites were determined using a monoclonal antibody (CL425: Coralie Munro, UCDavis, U.S.A.). The base-line concentration was 0.48±0.18 ug/g of wet feces and sustained values over 3.5 ug/g were considered pregnancies. The gestation period was 168±3 days and the luteal phase 15±2 days. First pregnancies (n=6) occurred after 553±19 days from birth. The birth season occurred manly in March (53.57%; rainiest month), April (29.76%) and May (9.52%). February (3.57%), June, October and December had few births (1.19% for each one). The non-invasive monitoring of the ovarian activity was important to determine the reproductive parameters of this specie and to plan the reproductive management of prepubertal and pregnant females in this captive population.



Abstract #82

Fecal testosterone monitoring in eastern diamondback rattlesnakes (*Crotalus adamanteus*).

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Topic: Reproduction

Presentation type: Poster

Abstract:

Eastern diamondback rattlesnakes (EDB) are a species sparsely distributed in their native range across the southeastern United States. Wild populations would benefit immensely from reintroduction of captive-bred individuals into native habitats, but a better understanding of their reproductive biology is needed. In this study, fecal samples from captive adult males ($n=3$; 140 ± 3.7 cm) and captive adult females ($n=2$; 149 ± 3.8 cm) were collected opportunistically over 11 months to investigate potential sex and seasonal differences. Fecal samples (0.5 g) were extracted using 90% ethanol, diluted 1:20 and 1:8 for males and females, respectively and fecal testosterone metabolites (FTM) measured using the R156/7 antibody and HRP conjugate (University of California, Davis, CA, USA). Parallelism between serially-diluted fecal extracts and standard testosterone hormone preparations was demonstrated for both sexes. Recovery of FTM from pooled samples was 104% ($r^2=0.99$) and 97% ($r^2=0.99$), for males and females, respectively. Mean FTM concentrations were 222 ± 83 ng/g for males and 78 ± 26 ng/g for females. For one adult male where 7 consecutive samples were collected 2-4 weeks apart, seasonal differences were measured with concentrations of 93 ± 9.7 during non-breeding season (Jan - April) and 412 ± 106 ng/g leading up to breeding season (May - Jun). It is important to note that hormone values for each fecal sample were not adjusted for number of days between defecation, and this is an area for further refinement for this species. These preliminary data indicate that fecal testosterone might be appropriate for measuring seasonality in adult male EDBs and has possible potential for distinguishing between adult males and females in this species.



Abstract #83

Herd-size, lactational-status and landscape affect stress status of free-ranging adult female Asian elephants: variations in fecal glucocorticoid metabolites

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Topic: Stress

Presentation type: Oral

Abstract:

Eco-variations and increasing anthropogenic activities in natural habitat adversely affect the well-being of free-ranging Asian elephants, the flagship species for conservation in India. Previously, we showed that variations in seasonality, animals' body condition and gender influence the stress-status of free-ranging Asian elephants, reflected by changes in the fecal glucocorticoid metabolite (fGM) levels. Here, we investigated whether or not animals' herd-size, their lactational status and the types of landscape influence the stress status of female Asian elephants. We conducted the study in seasonally dry tropical forests of Mysore and Nilgiri Elephant Reserves in southern India during February 2013 to December 2015. We collected 243 fecal samples from 191 identified adult female elephants to measure fGM contents by employing a well-standardized EIA, using a group-specific 11-oxoetiocholanolone EIA (lab-code: 72T with 11-oxoetiocholanolone-17-CMO:BSA as an antibody and biotinylated-11-oxo-aetiocholanolone as a label). We assessed the effect of herd size/lactational status and the landscape on the stress status of animals (n = 145 and 98). Our results showed that there was a negative association between herd size (range: >1 to 23 individuals) and fGMs levels (range: 1.07 to 0.35 µg/g; p <0.01, GLM). Moreover, in the lactating females (n = 24), the fGM levels were higher (1.38 µg/g) than that (0.67 µg/g) observed in the non-lactating females (n = 121). Interestingly, females (n = 145) in forested-landscape exhibited higher levels of fGMs (0.7 vs 0.43 µg/g; p <0.05; GLM) than those (n = 98) in human-dominated landscape. These results indicate that the investigated parameters such as herd-size, lactational status and type of landscape significantly influence the stress status of free-ranging Asian elephants, reflected by the levels of fGMs. The detailed analysis of herd-dynamics, endocrine status and types of human disturbances on ecosystem could provide an improved understanding on the stress-status of free-ranging female Asian elephants.



Abstract #84

Patterns of estrogen metabolites and color change in female white-cheeked gibbons

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Topic: Reproduction

Presentation type: Poster

Abstract:

Gibbons in the genus *Nomascus* undergo a series of color-changes as they mature. Infants are born blonde, and turn black between 12 and 18 months of age. Females revert to blonde again when they reach sexual maturity, generally between 6 and 9 years. While the triggers of color change have not been formally described, the assumption is that it is hormonally-mediated. Previously, we demonstrated that females chemically contracepted via MGA implants showed marked delays in completing their color change. Levels of fecal progestogens increased somewhat as females proceeded through color change, though the timing of color change was variable. Here, we report additional data on patterns of estrogen metabolites to more clearly elucidate possible underlying triggers to color change. We used and validated kits from Arbor Assays to measure E1G from fecal samples collected for three-month periods of time from females who had not yet completed their color change. We assayed samples from six females (one of whom was contracepted with an MGA implant) to explore patterns of E1G, and found significant differences across females. For 3 females, the majority of samples fell below the detection limit of the assay. The contracepted female, who was also the oldest female in the study (9+ years), had the lowest average concentration of E1G (0.28 ± 0.07 ng E1G/g feces). The youngest female, under age 3 and prior to any start of color change, averaged 0.80 ± 0.11 ng/g. The relationship between age and E1G was somewhat inconsistent however the oldest non-contracepted female (9 years) showed the highest hormone concentrations (2.97 ± 0.26 ng/g) and showed a marked increase in E1G with age. These results confirm our previous findings that contraception impacts both hormone levels and the progress of color change, and provide preliminary support for a relationship between hormones and color change in white-cheeked gibbons.



Abstract #85

A pilot study of methods for measuring oxytocin in giraffe urine and its potential as an indicator of positive welfare

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Topic: Methodologies

Presentation type: Oral

Abstract:

Developing tools to measure positive emotions in animals is a necessary precursor for creating environments where they can thrive in the care of humans. Peripheral oxytocin is correlated with various affiliative behaviors in mammals, including infant care and social bonding. The goals of this study were to (1) develop methods for measuring urinary oxytocin in *Giraffa camelopardalis* using a birth for biological validation and (2) determine if oxytocin concentrations are correlated with affiliative behaviors already established as indicators of positive welfare. Observed behavior (115 hours) of giraffes (n=3) was compared to urinary oxytocin concentrations in 59 samples collected opportunistically for 10 weeks before and 13 weeks after the birth. Samples were collected by pipetting off the floor or by centrifuging urine off pine shavings. Oxytocin concentrations were quantitated by EIA (#K048, Arbor Assays, Ann Arbor, MI). Preliminary results indicated shavings caused assay interference. However, experimentally soaking urine in shavings did not significantly alter concentrations (paired t-test, $t_3=-1.22$, $p=0.31$) when samples were purified by solid phase extraction (SPE). The assay was chemically validated for SPE-urine via parallelism (ANCOVA: $F_{1,11}=2.37$, $p=0.15$) and recovery, which averaged 117.7% across three concentrations. Inter-assay variation using an in-house control was 5%. Oxytocin values were adjusted for urine dilution using creatinine concentrations (Brown et al., 2008). In a linear mixed model with individual as a random factor, oxytocin concentrations increased significantly following the birth ($F_{1,52.80}=4.75$, $p=0.034$). Weekly oxytocin averages were weakly correlated with received sexual behavior in the breeding female (Spearman correlation=0.46, $p=0.056$) but did not reach statistical significance for other behaviors. Most likely, the number of samples did not produce enough statistical power for linking hormone concentrations to specific behaviors. These results suggest oxytocin can be reliably measured in giraffe urine, but further testing is required to establish oxytocin as an indicator of positive welfare.



Abstract #86

Analysis of reproductive steroid hormones in captive two-toed sloths (*Choloepus hoffmanni*) using the automated immunoassay analyzer AIA-360®

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Topic: Methodologies

Presentation type: Poster

Abstract:

Since there is limited information related to the reproductive hormone profiles of the two-toed sloth, *Choloepus hoffmanni*, a study was conducted in captive specimens at the "Sloth Sanctuary" (Cahuita, Limón, Costa Rica). The study was performed over a three month period, from November 2013 to January 2014, with a total amount of 269 fecal samples and 18 blood samples collected from five sexually mature females. The hormonal recovery from fecal samples was achieved by the ethanol boiling method, which showed in our conditions an 83% average recovery. The fecal extract was diluted (1:81) and measured in the fluorescent immunoassay analyzer AIA-360®; the results showed an intra-assay variation of 13.8% for progesterone and 9.4% for estradiol. Plasma samples received no previous treatment and were directly measured as indicated by the fabricant. The average concentration of progesterone in feces was 217.5 ng/g (CI95%: 132.3 – 302.6 ng/g), and 1704.0 pg/g (CI95%: 1597.3 - 1810.8 pg/g) for estrogen. In plasma, progesterone average values were 3.1 ± 0.9 ng/ml, and the estrogen levels were below the detection limit of the equipment (25 pg/ml). Although there was no strong statistical correlation between the fecal and plasmatic progesterone fluctuations, our data suggest that the plasmatic events are mostly reflected in feces two days afterwards. The average ovarian cycle duration was 24.8 days (12.3 - 37.3 days). Also, the levels of progesterone were elevated during the first half of November and, subsequently, showed a successive reduction in all the females tested, what suggests a seasonality pattern, where pregnancies occur during the rainy season and deliveries during the driest months. Our results demonstrate that fecal steroid extractions and their subsequent measurement in a AIA-360® allows the successful detection of progesterone and estradiol in *C. hoffmanni*, although it doesn't replace the plasmatic measurement to determine absolute values.



Abstract #87

How many males are enough? Feminization of green sea turtle foraging aggregations in the Pacific

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Topic: Misc

Presentation type: Oral

Abstract:

Wildlife populations are shaped by their environment and obtaining baseline information on how wildlife responds to environmental conditions is critical to identify physiological alterations. However, understanding climate impacts is complicated for marine species such as sea turtles where temperature directly regulates key physiological and behavioral parameters that, in turn, have significant consequences on population demography, habitat utilization, and human impacts. Warming may feminize sea turtle populations because sea turtle sex is determined by environmental conditions during incubation (temperature-dependent sex determination). Molecular tools can be used to elucidate these climate impacts; hormone analysis can determine demographic sex ratios at foraging grounds. Despite its potential, measuring testosterone (male turtles have distinctively higher plasma testosterone concentrations) to determine sex has been applied at surprisingly few sea turtle foraging locations. We previously validated an ELISA testosterone assay and described female biased sex ratios at three green sea turtle foraging locations in the Pacific [San Diego Bay and Long Beach, CA, USA; northern Great Barrier Reef (nGBR), Australia] with a greater bias towards females in the newer recruits to the population (immatures). We analyzed 3 additional foraging areas within the Pacific (Hawaii, Guam, and the Commonwealth of the Northern Mariana Islands) and preliminary results also show a female bias in these foraging grounds. Comparison of the present results to previous studies of sex ratios of immature green turtles at foraging grounds in the Pacific found nearly no bias in the early 1980s in Hawaii (1.0F:0.96M) or female-biased populations in the nGBR from 1987 – 1997 (3.2F:1.0M). Sex ratio data for foraging locations provide important baseline information for investigating the potential effects of climate change and consequential feminization of sea turtle population. Examination of operational sex ratios will be necessary to determine how many male turtles are enough to maintain sea turtle population viability.



Abstract #88

Assessment of Pseudopregnancy in American Black Bears (*Ursus americanus*) Through Hormone Analysis, Ultrasound Pregnancy Diagnosis and Body Temperature

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Topic: Reproduction

Presentation type: Oral

Abstract:

The American black bear (*Ursus americanus*) exhibits reproductive strategies highly synchronized with the environment to use energy resources efficiently. It is believed that black bears experience pseudopregnancy, a state in which non-pregnant females exhibit progesterone (P4) levels similar to gravid bears in absence of an actual pregnancy. However, this strategy conflicts with the efficient metabolism of black bears, as females with elevated P4 have higher body temperature to provide adequate environment for embryo/fetal development. Some studies have shown similar patterns in serum P4 profiles in parturient and non-parturient females, yet little focus was directed towards pregnancy diagnosis. Pregnant females experiencing in utero fetal death could display similar P4 levels to those producing cubs, thereby creating pseudopregnancy false positives. We tested whether black bears exhibit pseudopregnancy by comparing P4 and estradiol (E2) of females diagnosed by ultrasound as: pregnant producing cubs (P+C), pregnant not producing cubs (P-C), or not pregnant (NP). We collected 370 blood samples from 29 adult females (10 P+C, 9 P-C, and 10 NP). We validated and measured serum P4 and E2 using commercially available radioimmunoassays from MP-Biomedicals. Contrary to previous studies, P4 concentrations of P+C, P-C, and NP females were different ($P < 0.001$). Pregnant bears showed a 5-fold increase in P4 after embryonic implantation, while NP females maintained low P4. P+C and P-C females displayed lower E2 concentrations during pregnancy, while E2 of NP females did not change ($P > 0.05$). Rectal temperature of NP females was about 1°C lower than P+C and P-C females during gestation and hibernation ($P < 0.001$), consistent with NP females spending less energy wintering. Our results suggest that black bears do not experience pseudopregnancy as an adaptive reproductive strategy. Instead, miscarriage explains physiological changes of females previously thought to experience pseudopregnancy. Further research exploring embryonic death and other possible pseudopregnancy mechanisms is warranted in black bears.



Abstract #89

Interpreting statistical differences in bison (*Bison bison*) fecal glucocorticoid metabolite concentrations.

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Topic: Methodologies

Presentation type: Oral

Abstract:

Glucocorticoids play a critical role in the regulation of metabolism. Previously, seasonal changes in adult bison fecal glucocorticoid metabolite (FGM) concentrations were documented with values lowest in winter and highest in spring. Here, effects of body size, sex, ranch (n=3), and season were considered among yearling bison (n=30) sampled biweekly April—October. FGM were extracted from 0.2 g dry feces with 90% ethanol and measured using a corticosterone enzyme immunoassay (CJM006, University of California, Davis, CA, USA). Differences ($p < 0.05$) were detected in FGMs between large and small bison, ranches, and sampling dates, but not between sexes. $\text{Date} \times \text{sex} \times \text{size}$, $\text{date} \times \text{sex} \times \text{ranch}$, and $\text{ranch} \times \text{date}$ interactions were significant ($p < 0.05$). At one ranch, small males had greater FGMs than large males April—mid-June, but this relationship reversed late June—September. Small females had greater FGMs than large females for 7/13 sampling dates with no apparent pattern. Per sampling date, maximum differences in FGMs between ranches averaged only $0.78 \pm 0.19 \mu\text{g/g}$ (range=0.22-2.85 $\mu\text{g/g}$) with no ranch having consistently greater or lesser FGMs than the other ranches. It was not possible to distinguish a predictable metabolic advantage at any ranch. Differences in FGMs over time, however, were consistent across ranches, sexes, and sizes, and appeared to have a meaningful interpretation. FGMs were lowest in April ($1.04 \pm 0.02 \mu\text{g/g}$) before jumping to the highest concentrations May—July (range=3.31-4.16 $\mu\text{g/g}$), possibly corresponding to bison mobilizing energy from greening pastures. FGMs decreased again August—October (range=1.31-2.58 $\mu\text{g/g}$), likely as bison switched to energy conservation approaching winter. In this study, bison FGMs varied depending on the combination of physiological and environmental variables to which the animals were exposed at any given time, but season provided a comprehensive explanation for changes in FGMs that could enable future predictions about metabolism.



Abstract #90

Trials and tribulations of measuring fecal testosterone and glucocorticoid metabolites in male western lowland gorillas

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Topic: Methodologies

Presentation type: Poster

Abstract:

Several zoos house male western lowland gorillas (*Gorilla gorilla gorilla*) together in bachelor groups. The ability to longitudinally monitor fecal testosterone and glucocorticoid metabolites (FTM and FGM) using enzyme immunoassay techniques would provide useful information to correlate with observed developmental and behavioral changes, as well as to monitor group stability and well-being. Despite demonstrating parallelism and achieving good recoveries (>96%), analysis of 90% ethanol extracts from male fecal samples for FTM and FGM (using R156/7 and R4866 or CJM006 antibodies, respectively (all University of California, Davis, CA, USA)) revealed highly correlated (R156/7:R4866 $r^2=0.8$; R156/7:CJM006 $r^2=0.7$) hormone profiles, even among a bachelor group of immature males. These data suggested significant cross-reactivity of the testosterone and/or glucocorticoid assays with non-target metabolites and/or co-production of androgens and glucocorticoids by male gorillas. Samples were subsequently 1) hydrolyzed with B-glucuronidase/arylsulfatase, or 2) hydrolyzed and further extracted with petroleum ether and assayed using the R156/7 and R4866 antibodies. Parallelism was not achieved after the hydrolysis-ether extraction, and FTM and FGM profiles continued to demonstrate identical patterns ($p<0.05$, $r^2=0.8$) following hydrolysis extraction. HPLC analysis revealed that the CJM006 corticosterone antibody detected numerous androgen and glucocorticoid metabolites in male gorilla fecal extract (34% immunoreactivity aligning with glucocorticoid (GC) and 21% with androgen reference tracers), whereas the R4866 cortisol antibody detected cortisol almost exclusively (71% immunoreactivity aligning with GC and 3% with androgen reference tracers). Importantly, the R156/7 testosterone antibody detected dihydrotestosterone and other androgen metabolites in the fecal extract but also had some immunoreactivity with glucocorticoid metabolites (21% immunoreactivity aligning with GC and 54% with androgen reference tracers). These data suggest that the CJM006 antibody is not appropriate for measuring FGM in male western lowland gorillas, and the cross-reactivity of the R156/7 testosterone antibody with glucocorticoids within fecal extracts may result in inaccurate assessments of FTM.



Abstract #91

Monitoring individual differences in adrenal responses to varying handling regimens in African pygmy hedgehogs (*Atelerix albiventris*)

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Topic: Stress

Presentation type: Poster

Abstract:

The African pygmy hedgehog is commonly used in animal ambassador programs at zoos. A recent study found that handling frequency and number of years in the program was positively correlated with adrenal activity in hedgehogs, but there were significant individual differences, suggesting that some hedgehogs may be better suited to programs than others and that some animals may not be habituating to handling over time.

One theory is that offering choice or control to program animals may help reduce this stress response and increase animal welfare. In our study, we investigate these individual differences further by studying more animals longitudinally and by looking at how different presentation/handling options may influence overall adrenal activity.

Specifically, our objectives are:

1. Validate a fecal glucocorticoid assay through ACTH challenge in a male and a female hedgehog;
2. Characterize patterns of fecal glucocorticoid metabolite (fGM) excretion in several hedgehogs to determine individual responses to training and handling; and
3. Evaluate whether the use of "choice bins" in which hedgehogs can choose whether to be handled on one side or remain untouched on the other side is correlated with changes in fGM concentrations.

Keepers collected daily fecal samples from several hedgehogs (N=7) for one year and recorded handling types and times as well as information on each animal's reaction when "choice bins" were used. We analyzed all samples using the Arbor Assays cortisol EIA mini-kit.

Preliminary analyses show significant differences in average and baseline (+SEM) fGM concentrations between individuals, with females showing higher fGM concentrations than males. Average fGM concentrations range from 70.93 ± 1.93 ng/g to 290.53 ± 14.02 ng/g dried feces. The ACTH challenge resulted in a 1.6 to 2.2-fold increase over baseline 15-17 hours post-injection, which is similar to the fGM increase seen 1 day after programs involving handling.



Abstract #92

Measurement of feather cortisone in carmine bee-eaters (*Merops nubicus*): a noninvasive approach to measuring physiological health and well-being in birds

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Topic: Methodologies

Presentation type: Poster

Abstract:

Northern carmine bee-eaters (*Merops nubicus*; CABEs) are considered a desirable addition to zoos due to their striking appearance and conspicuous social and foraging behavior. However, because CABEs are challenging to manage and breed in captivity, they are rarely held in zoos. Few studies have looked into proximate or ultimate biological factors behind these challenges. Our objective was to develop and validate methodology to noninvasively evaluate health and well-being in CABEs by monitoring glucocorticoids and their metabolites. In birds, corticosterone and cortisol have primarily been quantified in feathers and feces, though evidence suggests their metabolites, especially cortisone, may be more appropriate. Molted feathers were collected from a colony of 37 CABEs aged 1-21 years. Samples were separated into pools of 5 feathers and chopped into <5mm pieces. Chopped feather pieces were either extracted overnight (approximately 16 hours) and at room temperature (21-24 degrees Celsius) in 100% methanol or pulverized using a homogenizer before extraction under the same conditions. While corticosterone [C. Munro, UC Davis, CA, Antisera CJM006; 14.25% cross-reactivity with desoxycorticosterone] was barely detectable and could not be validated from feather extracts, feather cortisone [Arbor Assays, Cat. No. K017-H5; 31.7% cross-reactivity with 5 α -dihydrocortisone] was readily measurable. Serial dilutions of both chopped and pulverized feather extracts were parallel to the cortisone standard curve ($P=0.403$ and $P=0.468$, respectively). Cortisone from pulverized samples was approximately four-fold higher than that from chopped samples. These results indicate cortisone may be the more appropriate biomarker for studying well-being in CABEs using feathers.



Abstract #93

Chemical contraceptive impacts on cyclic progesterone and sexual behavior in captive Western lowland gorillas

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Topic: Reproduction

Presentation type: Poster

Abstract:

This study examined the behavioral and hormonal impact of oral contraception on captive female Western lowland gorillas (*Gorilla gorilla gorilla*). For regularly cycling mammals in North American zoos, chemical contraceptives can be used to reduce the likelihood of unintentional offspring, but there is not much known on how the addition of the contraceptive impacts social behavior. Sexual behavior occurrence data were collected on a 1/0 calendar as observed by keepers. The 1/0 calendar indicated observations of sexual behavior as either occurring or not during the day. Fecal samples were collected from four gorillas on three methods of oral contraception and were analyzed for fecal progestogen metabolites (FPM) using a validated enzyme immunoassay (0.1 Low-Ogestrel 28, 0.3 mg norgestrel, 0.03 mg ethinyl estradiol daily; 0.2 Nortrel 1/35, 1 mg norethindrone, 0.035 mg ethinyl estradiol daily; 0.1 Norethindrone 0.35 mg daily). FPM levels varied from individual depending on the oral contraceptive brand, but the study population averaged 52.45 ± 31.01 ng/g. This is compared to the average FPM concentration of 221.28 ng/g in a representative, non-contracepted and normally cycling female gorilla. This difference was significant ($p < 0.001$). Significant differences in average metabolite concentration were discovered to be contraception brand dependent, with average FPM concentrations ranging from 30.86 ng/g to 76.88 ng/g ($p < 0.001$). The results indicate that all methods of oral contraception included in our study altered hormone concentration and patterning. Sexual behavior observations appear to be codependent on progestin fluctuations and social cues from other females in the troop. This codependency was demonstrated as a reduction of sexual behavior observations in the contracepted females when compared to a normally cycling female living in a troop with other normally cycling females. This study demonstrates the importance of considering troop dynamics and chemical contraception type before making animal management decisions in a zoo setting.



Abstract #94

Determination of reproductive state in female Hartmann's mountain zebra (*Equus zebra hartmannae*) using fecal progesterone and estradiol

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Topic: Reproduction

Presentation type: Poster

Abstract:

Little is known about the reproductive endocrinology of the Hartmann's mountain zebra (HMZ). For many *Equidae*, single blood or urine samples can be used to detect pregnancy however, HMZ's at Disney's Animal Kingdom Lodge® are housed in semi-naturalistic environments, making urine collection difficult. For a non-invasive hormone monitoring method, we collected fecal samples to characterize estrous cycles and pregnancy to help facilitate AZA species survival plan (SSP) goals.

A total 2,440 fecal samples were collected 3 to 4/week (N=6 females; 2011-2015), extracted with 80% methanol, and stored frozen prior to enzyme immunoassay. Serial dilutions of extracts gave displacement curves parallel to the progesterone (P4) and estradiol (E2) (monoclonal CL425; rabbit polyclonal R0008; C.J. Munro, UC Davis, CA) standard curves.

Ovarian cycle P4 peaks were variable ranging from 150 to 1,000 ng/g, whereas fecal estradiol had no observed cyclic pattern or peaks. Cycles averaged (\pm SD) 22.48 \pm 6.8 days, (N=71; range 14-30). At 17 weeks post-conception, there is a 1 to 8-fold increase in fecal E2 from baseline concentrations (calculated by iteration), and a 2 to 15-fold increase (maximum 108-fold) in P4 relative to luteal peaks. Estradiol increased mid gestation reaching 24-fold higher than baseline. Gestation averaged 381.71 \pm 10.21 days (N=7; range 364-399). Two of seven pregnancies were monitored with only one sample per month. This monthly protocol was able to detect acyclicity, cycling, and pregnant post-week 17 increases in P4 and E2, allowing us to approximate conception date and parturition window.

Fecal samples collected 3 to 4/week can be used to monitor estrous cycles and detect pregnancy for future SSP goals that may include cycle synchronization and artificial insemination. Alternatively zoos can effectively monitor reproductive state of HMZ mares with monthly samples. This monthly protocol is a good alternative for facilities unable to collect blood or urine to monitor their population.



Abstract #95

Tracking patterns of the menopausal transition through endocrine changes in the common chimpanzee (*Pan troglodytes*)

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Topic: Reproduction

Presentation type: Lightning Talks

Abstract:

Chimpanzees—the best studied of the great apes and our closest living relatives—share with us similar ages of terminal female fertility. Yet, unlike humans, chimpanzees generally become infirm during their fertile years and rarely survive them in the wild. As a result, the effects of this general physiological senescence on ovarian function in the common chimpanzee (*Pan troglodytes*) have been understudied. We know very little of age-mediated reproductive endocrine changes through the thirties and beyond. This project improves the record by collecting and quantifying age-specific endocrine data along the chimpanzee hypothalamic-pituitary-ovarian [HPO] axis [n=19 individuals; mean age= 42.47 yrs], a regulatory system subject to age-mediated degradation, for comparison with published data on humans. We measured urinary Estrone-3-Glucuronide [E1G; DetectX® Estrone-3-Glucuronide (E1G) Immunoassay], Pregnanediol-3-Glucuronide [PdG; DetectX® Pregnanediol-3-Glucuronide (PDG) Immunoassay], and cortisol [DetectX® Cortisol Enzyme Immunoassay] via enzyme-linked immunosorbent assay [ELISA]. We also evaluated urinary Follicle Stimulating Hormone [FSH] levels using chemiluminescent immunoassay to derive beta subunit assay. Creatinine was analyzed using specific gravity measures. Preliminary results reflect a pattern of simultaneously suppressed ovarian steroid hormones and gonadotropins with advanced age in the species [Cloutier et al. 2010], which is in opposition to the typical human pattern of elevated gonadotropins coupled with suppressed ovarian steroid hormones after menopause. This, coupled with recent chimpanzee ovarian follicular decline data [Cloutier et al. 2015], are evidence of clear differences in reproductive aging between *Homo* and *Pan*. We may expect that changes in brain aging that evolved with increased longevity in the human lineage likely have consequences for the physiology of perimenopause in our genus. By collecting relevant hormone data in captive chimpanzees, we improve our understanding of aging in our sister genus as well as our own.



Abstract #96

Endocrine monitoring of pair bond strength in captive whooping cranes

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Topic: Reproduction

Presentation type: Poster

Abstract:

Whooping cranes (*Grus americana*) are a long-lived, monogamous species. Once a pair forms, they typically mate for life and build a strong pair bond. However in captivity pairs are formed based on genetic recommendations with limited allowance for mate choice. There is evidence that poorly producing or unproductive pairs may have a weak pair bond, leading the pair to act as social pen mates rather than a true reproductive pair. Oxytocin is known to be important for bond formation in many monogamous species through all classes of vertebrates, including fish (cinnamon clownfish), rodents (rats and voles), and primates (marmosets). In birds, oxytocin administration has been shown to increase arousal behaviors in cockerels, while a lack of oxytocin prevents pair formation in zebra finch. We sought to validate an oxytocin assay to allow further investigation of pair bond mechanics in whooping cranes with the ultimate goals of 1) increasing our understanding of whooping crane reproductive physiology and 2) creating a management tool which can determine the strength of pair bonds. Plasma samples were collected from all pairs on the same day, outside of the normal breeding season, during annual Fall health examinations at Patuxent Wildlife Research Center, in Laurel, MD. Pairs were selected based on reproductive performance in the previous breeding season (3 non-productive, 3 egg laying, 3 egg laying and raised a chick). A serum pool was extracted following recommended procedures and analyzed using the DetectX Oxytocin Enzyme Immunoassay from Arbor Assays. Results showed inadequate hormone recovery (roughly 34%) and failed linearity of spiked samples. Further validation of different serum extraction methods are necessary to determine if oxytocin levels differ between these pair classes and if oxytocin could be informative toward pair bond strength in this species.



Abstract #98

Fecal testosterone and aggression in the blue-billed curassow (*Crax alberti*)

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Topic: Stress

Presentation type: Poster

Abstract:

Blue-billed curassows (BBCs) are challenging to manage because of aggression towards keepers and conspecifics. Testosterone can mediate aggressive behavior and this study sought to investigate its role to help improve management. Feces collected from adult males (n=9) and adult females (n=9) 3/week for 12 months from 4 institutions (H, M, N, WO) were lyophilized, weighed (0.5 g) and extracted with 90% ethanol. H and WO maintained 4 and 3 pairs of BBCs, respectively and M and N each housed a single pair. Extracted feces were analyzed by EIA using testosterone antibody (R156/7) and HRP (University of California, Davis, CA). Dilutions yielded curves parallel to the standard curve with recoveries of 91%, $r^2=0.992$. Aggressiveness of the males on a scale of 1-4 was recorded throughout the year. Mean testosterone was examined between males using one-way ANOVA RM and aggression using one-way ANOVA on ranks. Similar concentrations of testosterone were measured for males in 3 institutions (range 219 ± 27.7 - 378 ± 36.7 ng/g), and 1 institution (WO) had lower concentrations (range 89 ± 7.4 - 161 ± 13.4 ng/g; $P<0.05$). Females at M and N had higher concentrations ($P<0.05$; 323 ± 80.8 ng/g and 396 ± 40.9 ng/g) than females where there were several birds (range 51 ± 3.6 - 138 ± 18.6 ng/g). One male was transferred from M to H and fecal testosterone decreased from 468 ± 69 ng/g to 179 ± 17 ng/g and aggression scores decreased from 2.6 ± 0.17 to 1.7 ± 0.09 . Further studies will investigate whether aggression might also be linked to environment and social pressures as well as testosterone.



Abstract #99

Circannual Thyroid Function in Seasonal Species

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Topic: Misc

Presentation type: Oral

Abstract:

Circannual changes in thyroid activity can drive seasonal changes in energy balance, reproduction and activity. This has been studied in few species because of difficulty in repeated blood collection, however non-invasive assessment of thyroid function via analyses of fecal tri-iodothyronine hormone (fT3) concentrations has been reported recently. The assessment of thyroid function could provide valuable insight into the physiological responses to captive management practices but it first has to be determined if the species in question have circannual patterns of thyroid activity. Our goal was to non-invasively determine seasonal changes in thyroid function in two threatened northern species, the polar bear and the Vancouver Island marmot, both of which have seasonal patterns of energy balance, reproduction and activity in the wild. Fecal samples were collected from adult male and female polar bears and Vancouver Island marmots housed in zoos in Canada. All animals were housed under natural photoperiod. Samples were collected daily throughout the year from polar bears and during the non-hibernating season of marmots. Samples were extracted with 80% MeOH and fT3 concentrations were assessed using an enzyme-immunoassay that was developed using a commercial antibody (Sigma T2777). Results indicated captive polar bears do not have any apparent seasonal pattern in thyroid activity. The average fT3 concentrations were 76.3 +/- 5.7 ng/g feces (range 35.4 to 163.3 ng/g). In contrast, fT3 concentrations showed a strong seasonal pattern in the Vancouver Island marmots, with significant ($P < 0.05$) monthly differences during the non-hibernating season. Concentrations were highest immediately after emergence from hibernation in April (37.0 +/- 1.6 ng/g), decreased throughout May (20.1 +/- 0.9 ng/g) and reached a nadir by July (5.2 +/- 0.4 ng/g). These results suggest that although both of these species are seasonal, the thyroid gland may play different roles in circannual patterns of reproduction, energy balance and activity.



Abstract #100

Factors affecting whole-body metabolism in the male giant panda

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Topic: Misc

Presentation type: Poster

Abstract:

The energy to reproduce can only be achieved once basic physiological needs are met. The giant panda is unique among mammals in that the primary energy source (bamboo) is very low in digestible energy. We sought to evaluate endocrine and environmental factors that affect metabolism in reproductive male giant pandas. For this study we evaluated the feces of eight male giant pandas for excreted thyroid hormone (triiodothyronine [T3; Sigma-Aldrich T-2777] and thyroxine [T4; HyTest 2T6 MAB XM212]) metabolites (determined by HPLC), as well as mean ambient temperature, photoperiod, and humidity. Throughout the annual reproductive cycle, T3 and T4 values correlated with each other in every male ($r = 0.38 - 0.79$; $P = 4.1 \times 10^{-17} - 8.6 \times 10^{-7}$). Additionally, a consistent seasonal pattern in thyroid hormone values was evident with excreted T4 ($H_4 = 78.5$; $P < 0.001$) and T3 ($H_4 = 151.9$; $P < 0.001$) greatest ($P < 0.05$) during the pre-breeding period (Oct. 1 – Jan. 31), declining as the breeding season progressed (Feb. 1 – May 31), and increasing again in the non-breeding period (Jun. 1–Sep. 30). Interestingly, these patterns in thyroid hormones were negatively correlated ($r = -0.30 - -0.22$; $P < 0.001 - 0.002$) with photoperiod and positively correlated ($r = 0.26 - 0.31$; $P < 0.001$) with humidity; however only T3 demonstrated a relationship with temperature ($r = -0.17$; $P = 0.0006$). These data indicate that the influence of reproduction and environment in the giant panda are intertwined in regulating metabolic activity. The increase in thyroid hormone values during the pre-breeding period conforms to previous descriptions of male giant pandas increasing body mass, testicular size and volume in preparation for the breeding season. These results establish normative patterns in reproductive male giant pandas and provide insight about the species utilization of energy to sustain reproduction.



Abstract #101

Reproductive characteristics of female waterbuck (*Kobus e. ellipsiprymnus*).

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Topic: Reproduction

Presentation type: Poster

Abstract:

The common waterbuck, *Kobus e. ellipsiprymnus*, a medium sized African antelope listed as Least Concern in the 2016 IUCN Red List of Threatened Species. Though well represented in protected areas throughout sub-Saharan range countries, the overall current population trend is considered to be decreasing. To support conservation and genetic management of the small captive population in Australasia, knowledge of basic reproductive physiology is a pre-requisite in the development of assisted reproductive technologies. This study was designed to characterise basic reproductive norms of female waterbuck in the Southern Hemisphere. Births of waterbuck in New Zealand in the past 30 years occurred throughout the year with the greatest number of births during summer months (14/21; 67%). The average interbirth interval was 344 ± 9.6 days (mean \pm s.e.m., $n = 14$ gestations). Ovarian cycle length was investigated by measuring progesterone concentrations (EIA CL425 antibody and P-HRP, UC Davis, Davis, CA, USA), in faecal samples collected non-invasively (2-5 samples per week) from three non-pregnant females during a five-year period ($n=700$ animal ZAA/68, $n=609$ animal ZAA/78 $n=722$ animal ZAA/80). Longitudinal patterns of progesterone concentrations indicated that non-pregnant females exhibited regular ovarian cycles throughout the year with a cycle length of 24.7 ± 0.21 days (mean \pm s.e.m., $n = 93$ cycles). Rapid luteolysis and ovulation was induced by i.m. administration of prostaglandin-F₂alpha analogue during the luteal phase. Two injections 12-15 days apart administered via dart minimised animal handling, and on-going studies are investigating the protocol in the application of transcervical artificial insemination. In the absence of a male waterbuck, oestrous behaviour was not always obvious but bunting, chin resting, head to tail circling, "Laufschlag" and mounting were commonly observed 48-52hr after prostaglandin. Bunting and mounting was also recorded during natural periods of nadir progesterone concentration, oestrous behaviour was typically observed less than a day.



Abstract #102

The ecological tipping point: wild cotton-top tamarins respond to ecological factors, habitat loss, and fragmentation with altered reproductive patterns and success

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Topic: Reproduction

Presentation type: Lightning Talks

Abstract:

Habitat loss, forest fragmentation, and climate change threaten many species and cause not only behavioral changes but also influence reproductive success. To understand the effects of ecological factors (food availability, rainfall, and temperature) on cotton-top tamarin (*Saguinus oedipus*) reproductive success, 15 years of hormonal, behavioral (feeding, group composition, intra- and intergroup aggression), and climatic (rainfall, temperature, and humidity) data were collected from our long-term study site in Santa Catalina, Colombia, a 300 ha forested area on privately owned land. As pregnancy does not always produce viable living offspring, hormonal assays on fecal samples were used to monitor conception, loss of pregnancy, and parturition. For endocrine analysis, 10,128 fecal samples were collected between August 1999 and October 2014 from 56 females (178.5 ± 326.2 samples/female; 590.8 ± 919.1 days; range 1 day through 12.9 years). Female reproductive status was determined using fecal estrone and progesterone metabolite enzyme immunoassays (antisera R522/R522-2; P70, C.J. Munro, UC Davis, CA; progesterone EIA Kit #K025, Arbor Assays). Females showed a strong seasonality in reproduction, with conception occurring during periods of low fruit availability and parturition correlated with seasonal rainfall and high fruit availability. Variation in yearly rainfall was not a strong predictor of pregnancy loss nor the number of offspring produced. The proportion of conceptions that ended in pregnancy loss has increased in recent years, suggesting that amplified ecological pressures combined with habitat loss, changes in population density, and availability of resources have created an ecological tipping point that negatively influences reproductive success in cotton-top tamarins. These findings support the need for conservation efforts that protect remaining forests, restore and reforest areas, and reconnect forest fragments to increase the population size of this critically endangered species.



Abstract #103

Are zoo-housed collared anteaters (*Tamandua tetradactyla*) experiencing well-being?: seasonal assessment of basic health and serum cortisol

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Topic: Animal health and welfare

Presentation type: Lightning Talks

Abstract:

Zoo animal welfare is more commonly assessed based on the environment than directly measured in animals. Although most zoo-housed animals are under controlled feeding management, other environmental factors (such as temperature and photoperiod) may affect welfare measurements. Interpreting measurements without considering possible seasonal variations could thus lead to erroneous conclusions. In the present study, serum biochemistry, hematology, body weight and temperature, and serum cortisol were seasonally evaluated in 6 (3 male, 3 female) individually housed *Tamandua tetradactyla* from Córdoba Zoo (Argentina). Animals were exposed to natural temperature and photoperiod, and fed daily with a standard food mixture. Environmental enrichment was performed twice a week. Individual blood samples were collected in autumn (May), winter (August), spring (November) and summer (February). Considering that several wildlife studies show that glucocorticoids increase after 2-5 minutes from capture, blood was obtained before 3 minutes (physical restriction) in the morning from the coccygeal vein. Serum cortisol (ng/dL) was measured employing electrochemiluminescence immunoassay (Roche Diagnostic; previously validated). Generalized linear mixed model analysis were applied and LSD Fisher test. Seasonal changes in: creatinine (winter<others seasons; $p=0.02$), total protein (autumn < winter and summer < spring: $p=0.009$), albumin (autumn < others seasons; $p=0.005$), and percent eosinophils (range: 0-4%). No seasonal changes (mean \pm SD) were found in body weight (7.9 \pm 1.4 kg), temperature (33.9 \pm 0.8 °C), or cortisol concentrations (33.2 \pm 13.7). Statistical analysis revealed differences in cortisol according to sex (male: 41.3 \pm 11.3 > female: 18.1 \pm 7.4; $p=0.01$). This study did not reveal seasonal differences in most welfare measurements. Observed values were within the range previously reported for this species, indicating that animals were healthy throughout the study period. The serum cortisol range could be a first (gender-dependent) reference value for assessing individual well-being. Finally, this study contributes not only to individual health monitoring but also provides relevant information for conservation programs for these species.



Abstract #104

VALIDATION OF NON-INVASIVE STRESS HORMONE MONITORING IN TROPIDURUS SPINULOSUS, AN ENDEMIC LIZARD FROM ARGENTINA AND BRAZIL

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Topic: Methodologies

Presentation type: Poster

Abstract:

Tropidurus spinulosus is a lizard inhabiting Argentina and Brazil. Despite its wide distribution, its conservation status has recently been upgraded to vulnerable. Measurements of glucocorticoids can be used to assess adrenocortical activity and stress response to environmental challenges, which may be negatively affecting the status of populations. Thus, the purpose of this study was to validate a corticosterone enzyme immunoassay (EIA) for the analysis of lizard feces. Wild lizards were captured in Córdoba (Argentina) and transported to the laboratory. Animals (males=4, and females= 3) were exposed to treatments of ACTH (Day 8; 5IU/kg), dexamethasone (Day 20; 0.1mg/kg) and transport (Day 36; 24h-period in a black out bag). Fresh feces were collected daily and fecal corticosteroid metabolite (FCM; ng/g) were assessed by EIA with corticosterone antiserum (CJM006; C. Munro). Biochemical validation of the EIA was performed by demonstrating parallelism with the corticosterone standard. Kruskal Wallis test revealed differences in measurements according to sex ($P=0.01$) and individuals ($P=0.0006$). Changes from baseline were analyzed for 5 days during each treatment. Post-ACTH increments were detected (24h: 92.1 ± 16.5 ng/g or 72h: 105.9 ± 25.5 ng/g, individual differences). In contrast, no effect was revealed after dexamethasone administration, although a trend for reduction was observed. Four lizards exhibited decreased FCM after transport, while three lizards showed increased concentrations (change from baseline: 45 ± 7 and 110 ± 64 %, respectively). Based on these results, adrenocortical activity was stimulated by exposure to ACTH, showing a peak between 24-72h. Unexpectedly, dexamethasone did not inhibit adrenocortical activity; that the treatment dose and/or number of lizards should be increased and may improve results of the study. Although no overall differences were detected after transport, individual profiles perhaps are indicative of coping styles. Finally, the corticosterone EIA is biochemically and pharmacologically validated, showing biological relevance to support further studies focused on adrenocortical response to environmental factors.



Abstract #105

An evaluation of the relationship between serum testosterone and cortisol levels and *Toxoplasma gondii* infection in wild Eastern quolls (*Dasyurus viverrinus*)

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

Once widespread throughout south-eastern Australia, the Eastern quoll is currently undergoing a rapid decline in the island state of Tasmania, its last remaining refuge. Despite high *Toxoplasma gondii* infection rates in declining population sites, there is no evidence that infection reduces their survival. *T. gondii* is a protozoan parasite of felids that has significant implications for the health of wildlife, livestock and humans worldwide. *T. gondii* infection has been known to influence behaviour, such as decreased fear of predation by cats (the definitive host), allowing the parasite to complete its life cycle. The potential impact of *Toxoplasma gondii* infection on the reproductive traits of wild male Eastern quolls was examined. Male Eastern quolls ($n = 72$) were captured at three study sites during the breeding season (May, 2011-2013), and blood samples were tested for seroprevalence of *T. gondii*-specific IgG antibodies and hormone levels. Serum testosterone and cortisol concentrations were quantified by enzyme-immunoassays (antiserum testosterone, R156/7; cortisol R4866; Coralie Munro, UC Davis, USA). Serum testosterone levels were higher in *T. gondii* positive than negative males (2.24 ± 0.22 vs 1.52 ± 0.18 ng/mL; $p < 0.05$) but there were no differences in serum cortisol levels (24.00 ± 1.22 vs 23.88 ± 1.73 ng/mL; $p > 0.1$). Testicular volume (2.11 ± 0.13 vs 1.63 ± 0.07 cm³; $p < 0.05$) and body condition index were also greater in *T. gondii* positive males (1.04 ± 0.02 vs 0.96 ± 0.02 ; $p < 0.05$). The positive relationship between *T. gondii* infection and sexual characteristics is interesting but it is unknown if the infection is a reflection of a trade-off between sexual traits and life history decisions or immunity or if *T. gondii* directly influences testosterone production. In the quoll, if *T. gondii* infection influences testosterone production, this may modulate quoll behaviour to selectively benefit the parasite, increasing transmission.



Abstract #106

Assay validation of stress hormones obtained non-invasively in skin swabs and urine from the Colorado boreal toad (*Anaxyrus boreas boreas*) and Mississippi gopher frog (*Lithobates sevosus*)

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Topic: Stress

Presentation type: Poster

Abstract:

Captive amphibian breeding programs are imperative to species survival, yet artificial environments contribute to stress leading to unknown effects on reproduction, survivability and health. We compared three commercial ELISAs for detection of stress hormones from skin swabs and urine collected from Colorado boreal toads (CBT; n=2) and Mississippi gopher frogs (MGF; n=2) in conjunction with an ACTH challenge. ACTH was administered intraperitoneally at 0.446ug ACTH g⁻¹ bodyweight in a 100ul 0.9% NaCl solution. Skin swabs and urine were collected in parallel: Day -3 to 0 (baseline) samples collected daily; Hours 2 to 24 (early ACTH challenge) swabs collected every 2 hours and urine collected every 4 hours; late ACTH challenge samples were collected at 36 hours and then daily (day 2 to 6). Aliquots from swabs (stored in 70% ethanol) were dried and resuspended (1:1) in 500ul PBS. Urine from the CBT, and skin swabs from both species, were analyzed using the DetectX® Cortisol and Corticosterone Enzyme Immunoassay (EIA) kits from Arbor Assays (AA), and the Corticosterone ELISA kit from Cayman Chemical (CC). Assay validation used pooled samples from 8-48 hour collections for each individual. Urine and skin swab hormone validations demonstrated parallel displacement with corticosterone standards, but not cortisol standards. The direct measurement of corticosterone was detectable at high (>300pg/ml) and low (<300pg/ml) concentrations using the CC ELISA, while the AA EIA only detected high concentrations; therefore, further analysis was conducted with the CC ELISA. In the CBT, corticosterone ranged from 57–1553pg/ml in swabs and 894–19769pg/mg Cr in urine. MGF skin swab corticosterone concentrations (72–703pg/ml), detected in the CC ELISA, were lower than CBT. In conclusion, the CC ELISA has a greater sensitivity when compared to both AA EIAs, and skin swabs and urine samples can be used to monitor stress in the CBT and MGF.



Abstract #107

Noninvasive monitoring of glucocorticoid levels in endangered cats in Russia

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Topic: Stress

Presentation type: Oral

Abstract:

Large cats (Amur tiger (*Panthera tigris altaica*) and Far-Eastern leopard (*Panthera pardus orientalis*)) are listed as endangered species in IUCN Red List. Although both subspecies live in China and North Korea the core part of their northern ranges is situated in Russia. Both subspecies habitats consist of extremely low winter temperatures (below -40°C) and a high snow cover. The aim of this study was to conduct non-invasive monitoring of stress levels over multiple years and to understand the effects of different factors on the animals' welfare in the wild. This study was conducted in 2010-2016 in four main regions of tiger habitat: "Leopard land" National Park (the only location where the Far-eastern leopard lives); State Ussuriisky Reserve (southern edge of the Amur tiger range); Sikhote-Alinskii Reserve (central range of Amur tiger) and Bastak Reserve (northwestern edge of Amur tiger range). We collected frozen or fresh feces samples in winter (species identification and defecation date were conducted based on footprints of large cats in the snow). Steroid extraction was conducted with 90% methanol. The concentration of glucocorticoid metabolites (GC) was determined with previously validated commercial kits (Immunotekh, Russia). All concentrations were calculated per 1.0 g of dry feces. We found that both air temperature and snow depth significantly affected GC concentrations in tigers (GLM: $F=5.9-1016$; $n=198$; $p=0.02-0.04$). The maximum level of GC (1440 ng/g) was coincident with a snow depth of 40-60 cm, although it was lower (668-1016 ng/g) when the snow depth was lower or higher. Photo-trapping data analysis showed that high snow levels, changes the activity patterns of tigers and their prey, possibly decreasing prey mobility and the tigers' energy expenditure resulting in a decreased average of GC levels in tigers. In leopard an increase of population density and tigers' numbers did not affected GC levels found in feces.



Abstract #108

Case Study: Non-invasive Assessment of Puberty and Pregnancy Status in African Lions

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Topic: Reproduction

Presentation type: Poster

Abstract:

Puberty and pregnancy status can be assessed in African lions using longitudinal fecal hormone analyses. However, this approach can be time-consuming and expensive. In this case study, our goal was to assess alternative methods of puberty and pregnancy determination in African lions maintained at the Cincinnati Zoo, specifically investigating urinary relaxin as a biomarker of pregnancy and evaluating hormonal responses to exogenous gonadotropins as an indicator of puberty. Serial fecal samples were collected from a paired adult female lion for progesterone metabolite analysis (CL425 monoclonal antibody, provided by C. Munro), and urine was collected at 73 and 74 days post-breeding for concentration by microfilter centrifugation and relaxin measurement using a bench-top kit (Canine and Feline Pregnancy Test Kit, Synbiotics Corp.). Elevated fecal progesterone levels at 60 days post-breeding were suggestive of pregnancy. Urine relaxin was readily detected in both samples, including after urine freezing and thawing, but not in a control sample collected post-pregnancy. Three female lion cubs were born at 113 days post-breeding and raised by the parental pair. Prior to initiation of contraception at 2 yrs of age, the cubs' puberty status was assessed by injecting two of the females with equine chorionic gonadotropin (600 IU) and monitoring fecal estrogens (R4972 polyclonal antibody, C. Munro). Both treated females had pronounced estrogen surges, whereas the third cub showed an apparently synchronous natural estrogen increase. Behavioral estrus was observed in two females, and all three females were subsequently placed on Ovaban. These preliminary results suggest that urinary relaxin testing is potentially useful as an indicator of pregnancy at mid-gestation in lions. Further, treatment of pubertal lions with exogenous gonadotropins can induce a pronounced estrogen surge consistent with ovarian responsiveness and reproductive maturity. Both of these methods require further testing for validation, but initial findings from this case study appear promising.



Abstract #109

Preliminary validation of a fecal glucocorticoid metabolite assay in seed-finch, *Sporophila angolensis*

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Topic: Stress

Presentation type: Poster

Abstract:

Seed-finch is widely spread through South America, however, most of population is kept in captivity for song competition purposes. Noninvasive stress monitoring would be an important tool to evaluate animals' welfare. We aimed to validate the species adrenal activity assessment using an adrenocorticotrophic hormone (ACTH) challenge and a glucocorticoid metabolite (GCM) assay. Five adult males were kept in individual cages at Universidade Estadual de Santa Cruz, Brazil and all droppings were collected at 4-h intervals during 24 hours, starting at 10a.m. Samples were kept frozen at -80°C for further processing (15 days). After thawing, samples were freeze-dried, pulverized, and ~0.1 g of dry sample was weighed and shaken for 30 min on a multivortex with one mL of 80% methanol. After centrifugation, the supernatant was recovered and stored at -20°C. GCM concentrations were measured by enzyme immunoassay (EIA), with a polyclonal antibody for corticosterone (CJM006; 1:15000) and corticosterone-HRP conjugate (1:70000; Coralie Munro, Davis, CA, USA). We used the parallelism assay to test for immunogenic similarity and determine the best extract dilution. The GCM concentrations in the pooled dropping extracts (diluted from 1:4 to 1:256) were not high enough to demonstrate parallelism with the standard curve, and it must be repeated. Nevertheless, individual samples run at 1:7 dilution with buffer assay gave an average of 64% of binding and GCM concentrations ranging from 54.9 to 237.3 ng/g droppings. In addition, a daily rhythm pattern in GCM excretion was detected with the highest mean (repeated measures ANOVA, $p < 0.05$) at 10a.m. (132.9 ± 12.9 ng/g) and the lowest at 10p.m. (55.3 ± 6.8 ng/g). The EIA seems to be suitable for GCM measurements in seed-finch and, with a few protocol improvements, we will proceed with the study to assess parallelism, matrix interference and response to ACTH challenge.

Key words: enzyme immunoassay, corticosterone



Abstract #110

Assessing welfare of zoo-housed cheetahs (*Acinonyx jubatus*) in different management roles using fecal hormone metabolites

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

Cheetahs are an iconic species serving multiple roles in the Association of Zoos and Aquariums (AZA) - in off-exhibit breeding facilities, on exhibit, as animal ambassadors, or in some combination of these management roles. However, the AZA cheetah population is not self-sustaining, and there is ongoing focus on increasing reproductive success and optimizing welfare in this species. In addition, the topic of ambassador animal welfare is a research priority throughout AZA, and cheetahs are an increasingly common ambassador species. Previous research has demonstrated that husbandry factors including housing, degree of human interaction, and individual temperament are associated with variations in behavior, fecal glucocorticoid metabolites (FGM), and health in multiple taxa. This study evaluated factors associated with behavioral and physiological measures of welfare in cheetahs housed under a variety of housing and management conditions, including animal ambassadors. Fecal samples were collected approximately every other day for 90 days from 71 cheetahs housed at 14 AZA facilities. Samples were lyophilized and extracted with 80% methanol. FGM concentrations were measured using a previously validated corticosterone-3-CMO-BSA EIA (IZW, Berlin, Germany) and were further validated in four cheetahs that experienced a housing change. Preliminary General Linear Mixed Model analysis revealed sex and age differences in FGM, but no association between enclosure size, social grouping, or opportunity to run on a lure course with FGM ($p > 0.05$ in all models). For exhibit cheetahs, those managed in protected contact had lower FGM than those managed in free contact ($p = 0.006$). However, there were no overall differences in FGM between cheetahs managed primarily on exhibit, as ambassador animals, or at off-exhibit breeding centers ($p > 0.05$ in all models), the latter two of which are managed exclusively in free contact. Additional measures of welfare, including behavior and temperament, are important to investigate before drawing conclusions about optimal management practices.



Abstract #111

Administration of biorelease progesterone and estradiol or GnRH induce estrous cycles and breeding in anestrous African white rhinoceroses (*Ceratotherium simum simum*)

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Topic: Reproduction

Presentation type: Poster

Abstract:

The aim of the study was to evaluate a single long-acting i.m. injection of progesterone (P4) and estradiol (E2) or GnRH in initiating reproductive activity in anestrous African white rhinos ($n=6$ females; $n=1-5$ replicates/female). Three experimental treatment groups varied by P4 dose (3000mg, Trt 1, 3; 4500mg, Trt 2) and needle length (1.5", Trt 1, 2; 3.5", Trt 3). One female from each treatment received 100mg E2 and $n=4$ females from Trt 1 and 2 received 250ug GnRH (Cystorelin or Factrel) during late follicular phase. Females were ultrasounded (Trt 1, 2) prior to (1-2 times/week for 10 weeks, during (day 0) and following (days 5, 11, 14, 17-30) treatment. Serum P4 concentrations (EIA, CL425, C. Munro) were low (0.69 ± 0.28 ng/mL) on day of treatment and increased by >2 ng/mL by day 2 post-injection. Peak P4 achieved in response to treatment varied by concentration and day (Trt 1, 11.9ng/mL, day 3; Trt 2, 12.3ng/mL, day 5; Trt 3, 41.6ng/mL, day 17). Regardless of treatment, females grew a pre-ovulatory follicle (3.14 ± 0.13 cm) by 22.8 ± 5.3 days post-injection. GnRH was administered at 3.27 ± 0.2 cm (range 3.01 - 3.6 cm) or E2 was given at 3.4 cm follicle size with ovulation confirmed 48 hr post-injection. No follicle measurements were obtained for Trt 3, but estrus and breeding were observed ($n=1$ female) days 23-24 post-P4 injection. Two of three females administered E2 exhibited estrus, bred, ovulated and resumed spontaneous reproductive activity. Subsequent estrous cycles occurred at either 31.3 ± 4.0 ($n=7$) or 61.7 ± 10.8 ($n=7$) days post-GnRH. Without GnRH or E2, follicles grew >3.8 cm and did not ovulate. Administration of a single injection of long-acting P4 stimulates growth of a pre-ovulatory follicle capable of successful ovulation in this species and the addition of E2 at late follicular phase can promote estrus and breeding.



Abstract #112

Measuring sex pheromone in an aggregation of common carp: a novel non-invasive way to locate invasive fish

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Topic: Aquatics

Presentation type: Oral

Abstract:

Measuring sex pheromone in an aggregation of common carp: a novel non-invasive way to locate invasive fish

Information on detection and abundance measurements of invasive species plays a key role towards their efficient management. Such information can be derived from measuring the biomarkers present in the natural environment. Most species of fish release species-specific chemicals into water, which act as pheromones and these pheromones can, therefore, serve as a biomarker for the species. Although several pheromones have now been identified in fish, these cues have not yet been measured in the natural environment. In this study, the release of a female sex pheromone (prostaglandin F₂alpha, PGF₂alpha) was measured for the invasive common carp (*Cyprinus carpio*) in the field. For eDNA and PGF₂alpha analyses, 14 samples were collected during the test phase (in presence of common carp) and another 14 were collected during the control phase (in absence of common carp). We used high resolution mass-spectrometry and UHPLC methods to measure PGF₂alpha and analyzed if the pattern of PGF₂alpha concentrations matched with the abundance of wild common carp. We also measured the concentrations of another established biomarker, environmental DNA (eDNA) in the water samples and matched its pattern with the concentrations of PGF₂alpha (correlations cannot be carried out due to differences in units of measurements). Concentrations of both PGF₂alpha and eDNA were at or below the detection limit (eDNA: 10 copies/ml; PGF₂alpha: 3.8 ng/ml) in the absence of common carp; however, both increased dramatically within large aggregations of carp (separate one-way ANOVA for eDNA and PGF₂alpha, $P < 0.05$) and reached final concentrations of 106 ng/ml and 590 copies/ml, respectively. Thus, this study proposes the use of pheromone measurements as a novel non-invasive way to locate and measure the abundance of aquatic invasive species, which will greatly aid in the management of the species in the open waters.



Abstract #113

Physiological Responses of American Toads to Artificial Light at Night

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Topic: Stress

Presentation type: Oral

Abstract:

Over 20% of the earth's surface is affected by Artificial Light at Night (ALAN). Exposure to ALAN disrupts ecological interactions and individual physiology across taxa. Most studies have not investigated the long-term effects of ALAN or its effects across life-stages. Amphibians are facing drastic, global declines caused by anthropogenic disturbances; however, few studies have focused on the effects of ALAN on amphibians, particularly long-term effects across life-stages. Amphibian's biphasic life-cycle suggests the effects of ALAN may not only affect the current life-stage but may also carry-over to affect later life-stages. We validated commercially available radioimmunoassays and tested for effects of ALAN and predators on American toad's (*Bufo americanus*) whole-body corticosterone (MP: Catalog #07-120102) and melatonin (IBL: Catalog #IB88111) production using a 2x2 factorial design across 40 artificial ponds. After metamorphosis, 10 toads from all 20 predator-free artificial ponds were randomly placed in laboratory terraria where they were exposed to either ALAN or natural light treatments (5 toads/treatment; 200 total toads).

Melatonin production per gram body weight increased between larval (4.41 ± 0.52 pg/mL) and post-metamorphic stages (23.16 ± 3.7 pg/mL), and in relation to body mass. Melatonin tended to be lower in metamorphs ($P=0.10$) and juveniles ($P=0.07$) when exposed to ALAN. Although juvenile exposure to ALAN was not related to corticosterone concentrations ($P=0.18$), carry-over effects of larval stage ALAN exposure (348.7 ± 53 pg/mL) significantly increased corticosterone production ($P=0.02$) compared to toads with dark nights (221.8 ± 34 pg/mL). Expanding our experiment to post-metamorphic life-stages revealed a carry-over effect of ALAN that is suggestive of long-term stress effects in amphibians. Artificial lighting applies to both free-ranging and captive programs for nocturnal and diurnal species. Knowing what effects light has on species' physiological health, especially threatened or endangered species, will be crucial for adapting outdoor lighting around natural areas and zoo housing to conserve and enhance a species' wellbeing.



Abstract #114

The Impact of Translocation and Homing on Corticosterone Concentrations in the Turks and Caicos Iguana

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Topic: Stress

Presentation type: Oral

Abstract:

The Turks and Caicos iguana, *Cyclura carinata*, is critically endangered due to introduced mammals and habitat alteration. Inter-island translocations are a key conservation strategy however suitable islands for translocation are scarce. This study documented post intra-island translocation stress and homing on Big Ambergris Cay, Turks and Caicos Islands by gender (male vs. female), age (adult vs. juvenile), and season translocated (wet vs. dry) for 96 iguanas over four, six-week study periods. Subject movements were radio-tracked and at each capture event (initial capture, capture for translocation and post translocation), both baseline (under 3 min) and restraint-stress (30 min) blood samples were analyzed for corticosterone concentration by radioimmunoassay. Differences in baseline and restraint-stress corticosterone over time for adult subjects were examined by 3*2*2 mixed ANOVAs (capture time*age*gender*season) with gender, age and season as between subject factors and capture time as a within subjects factor. Adult subjects exhibited significant increases in baseline and restraint stress corticosterone concentrations over the capture periods, $p < .001$ and $p = .004$ respectively. Female restraint-stress corticosterone concentrations were significantly greater than male concentrations across both seasons, $p = .001$. There were significant impacts of homing ability on post-translocation stress measures. Subjects that homed to their original capture location exhibited lower average baseline and stress-induced corticosterone concentrations overall, $p = .003$ and $p = .049$ respectively. Findings indicate that homing was perceived as stressful to the subjects and resulted in chronic stress. Baseline corticosterone concentrations increased over time and remained elevated, but did not reach initial restraint-stress concentrations. Animals also demonstrated the ability to mount stress responses throughout the study period with no signs of downregulation. The results of this study provide a systematic test of the effects of homing and post-translocation stress and can be utilized to develop translocation methods that promote iguana-friendly development throughout the Caribbean.



Abstract #115

Validation of a novel method using whiskers to track reproductive and stress-related hormones in Steller sea lions and northern fur seals

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Topic: Methodologies

Presentation type: Oral

Abstract:

Keratinized tissues have proven ideal for acquiring a record of physiological parameters, such as dietary stable isotope signatures. Unlike other tissues that provide a snapshot of hormones, whiskers may track reproductive histories for multiple years if hormones are incorporated as whiskers grow and concentrations vary with reproductive status. Steller sea lion (SSL; N=12) and northern fur seal (NFS; N=12) whiskers were obtained from live-captured animals during field research, bio-sampled carcasses, and animals housed in aquarium. Whiskers were sectioned with a hand chisel (0.25 – 1cm), pulverized, and steroid hormones extracted with 100% methanol at room temperature and rotation for 24 hours. Standard methods including recovery of added mass, parallelism and dilution linearity were used to validate enzyme immunoassay kits (Arbor Assay K003-H, K025-H, K030-H) for cortisol, progesterone and 17 β -estradiol. Progesterone, 17 β -estradiol, and cortisol were detectable in serial sections of SSL and NFS whiskers and multiple hormones could be measured from the same methanol extract. Progesterone detection required less whisker tissue compared to estradiol and cortisol. Therefore, whiskers were sectioned based on the mass requirement for progesterone and extracts from multiple sections were combined to measure 17 β -estradiol and cortisol. Cortisol concentrations were highest near the root and rapidly decreased towards the tip of whiskers (SSL: 6.6-106.9pg/mg; NFS: 0.4-167.8pg/mg). Whiskers collected from females with known reproductive histories were used to compare hormone concentrations during reproductive events including full-term pregnancy and estrous without pregnancy. Whiskers showed cyclical patterns in progesterone concentrations (SSL: 3.3-136.9 pg/mg; NFS: 48.6-1193.0pg/mg) along the length of the whisker which appears to signify previous pregnancies or luteal phases. However, estradiol concentrations (SSL: 0.25-0.77pg/mg; NFS: 7.5- 47.1pg/mg) did not show the expected patterns. These results indicate whiskers retain reproductive hormones throughout the length of the whisker and possibly give insights into multi-year reproductive histories of SSLs and NFSs.



Abstract #116

A fast high-performance liquid chromatography method as a potential tool for assessing fecal estrogen concentrations in jaguars (*Panthera onca*).

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Topic: Methodologies

Presentation type: Lightning Talks

Abstract:

In developing countries, the costs associated with antibody-based methods of hormone analysis can become a major limitation faced by researchers. A possible alternative is the use of high-performance liquid chromatography (HPLC) as it is a less costly method for quantifying hormones, pending thorough validation. In this study, we developed and validated an HPLC method to detect and quantify steroid analytes present in fecal samples of female jaguars (*Panthera onca*). Analytes with the same retention times as the standards estradiol, estrone, and progesterone were detected, but not in amounts high enough to allow for quantification. One particular analyte was named A2 because its retention time (6.7 min) was relatively close to that of estradiol (E2, 7.2 min). Concentrations of A2 were quantified via HPLC and classified as “baseline” or “elevated” according to an iterative process of calculation using fecal samples collected from two female jaguars during 3-4 consecutive months. Fecal estrogen concentrations were also assessed via enzyme immunoassay (EIA) using the estrone 3-glucuronide antibody (R522-2) and were classified as “baseline” or “elevated”. Because of the inherent differences between the two methodologies, concentration values of A2 (by HPLC) and fecal estrogens (by EIA) were not directly compared; instead, comparisons were made by checking how each sample was classified by each method and the percent agreement between them, which was ~90% for “baseline” and ~60% for “elevated”. It is possible that A2 is an E2 metabolite, but further studies are needed to confirm its chemical structure. If confirmed that A2 is indeed a major E2 metabolite, HPLC could potentially be used as a fast method to assess fecal estrogen concentrations in jaguars as it elutes within the first seven minutes of analysis; moreover, the percentage of samples with baseline versus elevated concentrations of A2 might help distinguish “flatliners” from “reproductive active” females.



Abstract #117

Monitoring reproductive status of female tarsier (*Tarsius tarsier*) based on fecal steroid metabolite measurements

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Topic: Reproduction

Presentation type: Oral

Abstract:

The spectral tarsier is one of the smallest living primates worldwide. The wild population is declining and attempts to breed the species in captivity have been limited success. One possible reason for this is that information on the reproductive biology of *T. tarsier* is extremely limited and data on the species reproductive physiology are completely lacking. We validated fecal estrogen and progestogen measurements for monitoring female reproductive condition (i.e. ovarian cycle, pregnancy) and to use this approach to provide the first data on cycle and gestation length based on endocrine information. Three females housed in pairs at the Primate Research Center of Bogor Agricultural University, Indonesia, were used in this study. Individual fecal samples were collected every second day in combination with observations on sexual behaviour for 15 months. Estrogen and progestogen metabolites were measured by enzymeimmunoassay using self-made antibodies raised against estradiol-17beta and 5alpha-pregnane-3alpha-ol-20-one, respectively. Hormonal profiles indicated that behavioural estrous was always associated with elevations in fecal estrogens followed by increases in progestogen levels indicating luteal function. Pregnancy was characterized by low levels of both hormones during the first month and markedly rising concentrations (estrogens: up to 18 fold; progestogens: up to 10 fold) during the remainder of gestation. Levels of both hormones decreased to low non-pregnancy concentrations within two days of parturition. Based on successive estrogen rises an average ovarian cycle length of 22.0 ± 5.7 (12-29) days (n=9 cycles) was found. Gestation length was 128d, 131d and 164d, the extended gestation ended by the death of mother and infant. The study demonstrates the validity of fecal hormone measurements for female reproductive monitoring in *T. tarsier*. The methods described offer the potential to facilitate the breeding management of the species in captivity and provide new opportunities for investigating basic questions of tarsier reproductive biology also in the wild.



Abstract #118

Validating New Methods for Quantifying Glycemic Response and Cellular Metabolism in African Elephants

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Topic: Animal health and welfare

Presentation type: Poster

Abstract:

Since 2010, poaching has led to the loss of 100,000 African elephants, 20% of the population. This crisis led to an increase in smaller reserves where individuals can be closely monitored. Our project focuses on establishing new methods to monitor nutritional- and health-related markers in managed populations of elephants. The first objective is to validate a new marker of cellular growth and metabolism, namely fecal thyroid hormone metabolites (fT3). Basal fT3 concentrations and body condition scores (BCS) will be determined in semi free-ranging elephants (n=6) from a private game reserve in South Africa and compared with fT3 concentrations and BCS from two free-ranging bull elephants which declined in BCS due to temporary injury. These samples were dried and extracted in 80% ethanol and stored frozen. Samples will be analyzed with a triiodothyronine enzyme immunoassay (#K056-H1, Arbor Assays, USA). Assay validation will be by serial dilution of pooled fecal extracts and % recovery of control spiked samples.

The second objective is to determine the variation in the six elephants' glycemic response by feeding the elephants different diets and monitoring the resulting blood glucose concentrations. On the morning of each trial, the elephant will be fed each of four different diets in a randomized sequence over a 4 week period: 1) Bana hay, 2) browse, 3) fruit, or 4) Bana hay w/glucose powder (10% of estimated daily caloric intake). Blood samples will be collected via the ear veins every thirty minutes until return to baseline (estimated to be three hours) and measured using a hand-held glucometer (Roche, USA). Diets will be analyzed for digestible energy, dry matter, crude protein, crude fat, neutral detergent fiber and acid detergent fiber. Body condition will be scored and % body fat measured with a modified A-mode ultrasound (Renco, Kansas, USA).



Abstract #120

Monitoring ovarian activity in jaguar (*Panthera onca*): determination of estrogen and progesterone fecal metabolite levels

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Topic: Reproduction

Presentation type: Oral

Abstract:

The major concerns for Jaguar conservation are increases in habitat loss, fragmentation and hunting. This species has been virtually eliminated from much of its historical range. With the severe decline of the wild populations, it has become increasingly important to understand the species' basic reproductive physiology for a successful conservation program.

We determined fecal estrogen and progesterone metabolite levels of a female jaguar maintained in captive conditions at the "Jaguar Experimental Breeding Center" from The Conservation Land Trust, in the wetlands of Ibera Natural Reserve, Corrientes, Argentina. A male was allowed to mate when the female showed external signs of estrus. Fecal samples were collected daily from April-December of 2016 (n=208). Fecal extracts (0.5g wet feces in 80% methanol, 24 h extraction at 4° C) were analyzed by enzyme immunoassay (EIA) using the Estradiol 17B-R0008 and Progesterone-R4859 antibodies (Coralie Munro, UC Davis, USA).

Mean duration of the ovarian cycle (defined as the number of days between two consecutive estrogen peaks) was 32.7±2.0 days (range: 28–37 days; n=4 estrous cycles). Mean estrous length was 6.2±0.4 days and luteal phase was 19.5±4.1 days, defined by a lapse of sustained high levels of progesterone (range: 11–27 days; n=4). Baseline concentrations of estradiol and progesterone were 9.5±0.5 and 137.1 ± 9.8 ng/g wet feces respectively. Peak fecal estradiol and progesterone concentrations reached values of 218.4±59.1 and 1330.1±113.0 ng/g wet feces, respectively.

The hormonal and behavioural data suggested that this female is polyestrous and that ovulation was not linked to the events of copulation alone, suggesting that spontaneous ovulation occurs regularly. This protocol was effective for monitoring the ovarian cycle in the jaguar, and the ovarian cycle duration and estrous length were coincident with the scarce data that exists on this species.



Abstract # 121

Testosterone in ancient hair from an extinct species

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Topic: Methodologies

Presentation type: Oral

Abstract:

Testosterone is a key regulator in vertebrate development, physiology, and behaviour. Whereas technology allows extraction of a wealth of genetic information from extant as well as extinct species, complimentary information on steroid hormone levels may add a social, sexual, and environmental context. Hair shafts have been previously used to sequence DNA from >50,000 14C years old Siberian woolly mammoths (*Mammuthus primigenius*). Hair-testing has also been used to measure endogenous steroids in multiple extant species. Here we measured testosterone from small quantities of hair samples from permafrost-preserved woolly mammoths dated to circa 10-60 thousand 14C years. Testosterone was extracted using methanol following our published hair-testing protocols, and quantified using a testosterone-specific antibody in a commercial enzyme-linked immunosorbent assay (ELISA) kit. The presence of testosterone in the mammoth hair samples was unambiguously verified via LC-MS/MS. Progesterone and dehydroisoandrosterone were also detected by the LC-MS/MS. Although we have too few individual samples for statistical tests (N=10), we found measurable testosterone in all mammoth hair samples, and sex differences in long-term integrated testosterone levels. Mean male hair testosterone level was twice that of females', with similar standard deviations. Our validated method opens up exciting opportunities to measure multiple steroids in keratinized tissues from extinct populations of mammals. This may be specifically applied to investigating life histories, including the extinct Quaternary megafauna populations whose remains are preserved in the permafrost throughout the northern hemisphere.



Abstract # 122

Characterizing the reproductive biology of the female pygmy hippopotamus (*Choeropsis liberiensis*) through non-invasive endocrine monitoring

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Topic: Reproduction

Presentation type: Poster

Abstract:

The pygmy hippopotamus (*Choeropsis liberiensis*) is endangered in the wild and experiences a number of reproductive issues under managed care in zoological facilities, including a high rate of stillbirths and failure of many pairs to reproduce. We evaluated reproductive cycles and pregnancy in the pygmy hippo using enzyme immunoassays (EIAs). Fecal samples were collected twice weekly for a one to three year period from 36 females housed at 24 zoological institutions. Fecal hormone metabolites were analyzed in three separate laboratories using established methanol- or ethanol-based extraction methods. Each laboratory used separate EIAs for progesterone and estrogen metabolites; results were compiled for analysis. Three progesterone metabolite EIAs (Pg-diol: 5b-pregnane-3a,20a-diol 3HS:BSA; PdG: pregnanediol-3-glucuronide R13904; mono-P4: Quidel clone 425) and three estrogen metabolite EIAs (E2a: estradiol-17b-OH 17-HS:BSA; E2b: estradiol 17b R0008; E2c: estradiol 17b R4972) accurately reflected reproductive events. Average estrous cycle length was 31.8 ± 7.4 days based on estrogen metabolite peaks and 30.9 ± 7.3 days based on nadir to nadir progesterone metabolite concentrations. Cyclical patterns were detected throughout the year, indicating a lack of seasonality. Estrogen metabolite peaks observed during pregnancy and lactation suggested follicular development may occur during both reproductive states. Pregnancy was most reliably demonstrated by an average 3- to 5-fold elevation in progesterone metabolites (Pg-diol or PdG) in the second half of gestation. Average gestation length was 203 ± 4 days for 15 pregnancies based on breeding to calving date. Our results provide valuable data for guiding long-term breeding management of the pygmy hippo and serve as a baseline for future studies addressing the potential influence of social structure, diet, body condition, and other husbandry factors on reproduction in this endangered species.

Results described in this abstract will be published in 2017 (Flacke et al., under review in *Theriogenology*).