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12-14 October 2015 - Berlin, Germany



Leibniz Institute for Zoo and Wildlife
Endocrinology

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We warmly thank our invited speakers for contributing keynote lectures:

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Curator of Reproductive Programs & Research, Toronto Zoo, Canada

Lee Koren
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5th ISWE CONFERENCE 12-14 October 2015



Leibniz Institute for Zoo
and Wildlife Research
IN THE FORSCHUNGSVERBUND BERLIN E.V.

PLENARY TALK

"INTEGRATED MATRICES REVEAL WILD VERTEBRATE LIFE HISTORY TRAITS"

Dr. Lee Koren - The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel. Lee.Koren@biu.ac.il



Life history traits contribute to individual fitness. They show diverse trade-offs between growth, survival, and reproduction. Steroids may be involved in the mechanisms mediating these trade-offs via participation in reproduction, growth, and development, or as coordinators, adjusting behaviours to circumstances and contexts. Their concentrations can therefore provide information to conspecifics (and researchers) on individual's physiological and social condition. While rapidly changing steroid levels reflect internal states, their lability presents a challenge to quantification. In the last two decades, this challenge led to the validation of non-invasive matrices that reflect longer time frames that are not influenced by the stress involved in capture and handling. Keratin-based matrices, such as hair, feathers, claws, and nails, are increasingly being used as a tool for quantifying endogenous steroids in wildlife and domestic animals. The main advantage of this approach is that it provides a long-term record of steroid hormone concentrations integrated over the period of growth. Since samples are unaffected by the momentary stress of collection, they are appropriate for studying long-term effects of stable social, physiological, nutritional, and environmental conditions, baseline levels, and chronic stress. Samples are easy to store and may be kept for long periods in paper envelopes at room temperature. Biological validations of hair cortisol and association of cortisol and testosterone concentrations in animal hair with those in saliva, blood, and feces have already allowed exploration of questions in behavioural ecology, cognition, conservation, ecology, and animal welfare. In this talk, I will focus on the opportunities that hair-, claw-, and feather- testing have opened, and the challenges that still persist. By integrating stress and sex steroid hormone concentrations, with data on aggression, communication, and affiliative behaviours, we study natural ecosystems, as well as environments that are affected by human disturbances. We study natural (e.g., predators, parasites, weather, food availability, disease, social conflict) and anthropogenic stressors (e.g., populations that are hunted, suffer habitat fragmentation, or are disturbed by environmental contaminations) in order to understand how individual life history traits shape fitness.





5th ISWE CONFERENCE
12-14 October 2015



Leibniz Institute for Zoo
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PLENARY TALK

“REPRODUCTIVE HORMONE ANALYSIS: DEVELOPING TOOLS TO UNDERSTAND POPULATION HEALTH”

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Reproductive hormones are important indicators of population health and growth, and thus, have been instrumental in helping wildlife biologists understand the dynamics of natural populations. In the past 30-40 years, intensive efforts, primarily due to the needs of conservation breeding programs, have resulted in the compilation of longitudinal reproductive hormone profiles from diverse species with daily or weekly samples collected over extended periods of time. The resulting hormone profiles provided detailed views of the reproductive system and gave some insight into phenomena that were difficult to observe in nature, such as reproductive dysfunction and senescence. In addition, knowledge of natural reproductive hormone patterns is a prerequisite for the development and application of techniques that manipulate reproductive outcomes, from ovulation induction to contraception.

In this presentation, I will discuss our role in providing Canadian wildlife biologists with the necessary tools to monitor, understand and implement solutions for the long-term sustainability of threatened species, focusing on two iconic Canadian species: caribou and wood bison. Caribou (*Rangifer tarandus ssp.*) are found across Canada and include diverse subspecies and ecotypes that inhabit specific niches from boreal forest to tundra. Anthropogenic impacts, including deforestation and natural resource extraction, have resulted in significant declines in population sizes. Current studies are investigating the use of reproductive hormone analyses to identify changes in population demographics that may help explain decreases in population stability. In contrast, wood bison (*Bison bison athabascae*), a genetically distinct subspecies of American bison, are localized in a specific region of open boreal forest in northwestern Canada. Present threats to the small remaining populations are due to the on-going risk of bovine tuberculosis and brucellosis originally transmitted from domestic cattle herds. Reproductive hormone analyses have formed the basis for understanding seasonality and reproductive output in this species in order to develop reproductive technologies to overcome the challenges of persistent disease. These studies will demonstrate how applied reproductive endocrinology can become a useful tool for the management of free-ranging wildlife populations.





Abstract #69

Excretion and measurement of corticosterone and testosterone metabolites in bank voles (*Myodes glareolus*)

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Topic: Stress

Presentation type: Oral

Abstract:

The bank vole (*Myodes glareolus*) is a commonly used model species in behavioral and ecophysiological studies. Thus, presenting a validated method for noninvasive monitoring of corticosterone and testosterone secretion is of high relevance. First, we performed radiometabolism experiments (n=6 in both sexes and steroids) to study metabolism and excretion of ³H-corticosterone and ³H-testosterone. Furthermore we evaluated the effect of circadian fluctuations and an ACTH challenge test on measured fecal testosterone (FTM) and corticosterone metabolites (FCM) in both sexes (12 animals each). Fecal samples were collected frequently (2h-4h intervals), homogenized and extracted with 80% methanol. Radioactivity in fecal extracts (and urine) was measured in a scintillation counter. FTM and FCM were analysed with a testosterone (measuring 17 β -hydroxyandrostanes) and 5 α -pregnane-3 β ,11 β ,21-triol-20-one (developed for mice) enzyme immunoassay (EIA), respectively. Males had significantly higher FCM levels than females and their main excretion route was via the feces (mean: 72%), whereas females excreted nearly equal portions in both excreta. For testosterone the main excretion route was via the feces in both sexes (~80%). The time course of excretion was similar in both sexes; urinary and fecal peak excretion was observed 2-8 h (first sample) and 4-10 h after injection. Additional, smaller peaks (after 12-16 h) probably reflected an enterohepatic recirculation of some metabolites. As shown by HPLC immunograms, both injected steroids were heavily metabolized. Several (more in case of corticosterone) metabolites were present in the feces and the tested EIAs reacted with some of them. Time of day had a significant effect on measured fecal steroid metabolites (more pronounced in FCM). As expected, males had significantly higher FTM levels than females. ACTH administration significantly (p=0.014) increased FCM values; peaks were observed 4-8 h after injection. In conclusion, both tested EIAs proved suited for a non-invasive measurement of glucocorticoids and androgens in bank voles.



Abstract #110

Using faecal glucocorticoid metabolites from wild animals as biomonitoring tools: Nile crocodile stress and freshwater health

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Topic: Stress

Presentation type: Oral

Abstract:

Physiological stress, measured using faecal glucocorticoid metabolites (FGMs), is used to test specific hypotheses about social and ecological stressors of wildlife. FGMs may be used as a biomonitoring tool for assessing ecosystem health. However, few studies have attempted to validate a broad-scale application of FGM techniques and no guidelines exist for selecting candidate species and for FGM-based biomonitoring approaches. We attempted to validate the use of Nile crocodile (*Crocodylus niloticus*) FGMs to monitor freshwater health in southern Africa. We conducted a biochemical validation of an enzyme-immunoassay detecting corticosterone metabolites (with a 11 β ,21-diol-20-one structure), a degradation study, and a prospective power analysis in captive animals. We then conducted a preliminary field study of 18 populations of wild crocodiles (1 to 55 samples per site, median 8) at sites across South Africa, where we also measured physico-chemical water parameters. Within-population variability for wild crocodiles (e.g. range, 0.10 to 1.85 ug/g dw) was large relative to variability within captive crocodiles (range, 0.13 to 0.65 ug/g dw). FGM levels remained stable over 14 days (fluctuating between a 0.01-fold and a 0.23-fold decrease), as crocodile faeces dried out. For FGMs in wild crocodiles, within-population variability (e.g. range, 0.10 to 1.85 ug/g dw) was large relative to among-population variability (population medians ranged from 0.07 to 1.29 ug/g dw). Putative baseline FGM levels for wild crocodiles were generally higher than baselines from captive animals (0.2 ug/g dw), even for pristine field sites (e.g. 0.52 ug/g dw). The highest FGM levels from wild crocodiles were approximately 8-fold higher than a putative baseline for captive animals and were from sites with degraded water quality. Using Nile crocodile FGMs as a biomonitoring tool for assessing freshwater health may be feasible, albeit providing a coarse metric. We offer suggestions for selecting candidate species and for guidelines for FGM-based ecosystem biomonitoring.



Abstract #91

Physiological evaluation of anthropogenic effects on hoary fox (*Lycalopex vetulus*) in the Brazilian Cerrado

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Topic: Stress

Presentation type: Oral

Abstract:

Nestled in central Brazil, the Cerrado is the largest savannah ecosystem in South America and is the home of one of the least studied canid species in the world, the hoary fox (*Lycalopex vetulus*). Intense agricultural and cattle ranching activities over the past 40 years has severely fragmented the Cerrado ecosystem and population trends of the hoary fox under these conditions remains unknown. The objective of our study was to compare adrenal hormone metabolite concentrations with behavioral observations to quantify and evaluate how anthropogenic activities were influencing the hoary fox and greater wild canid community. Fecal samples (n=211) were collected from a wild population of 24 radio-collared hoary foxes in human modified and fragmented Cerrado habitat in Goiás state, Brazil. The fecal samples were collected under three conditions: from box traps, witnessed defecation, and opportunistically. In addition, 8 individuals were observed on foot at night using a spotlight and binoculars for a total of 112 hours. Fecal glucocorticoid metabolite (FGM) concentrations were analyzed using a corticosterone polyclonal antibody (CIM006, Arbor Assays, Ann Arbor, MI) that was biologically validated with an ACTH challenge, HPLC analysis, parallelism, and recovery check. FGM concentrations from samples collected during captures (2.183 ± 0.28 ?g/g) were significantly greater ($p < 0.05$) than those collected as observed defecations (0.807 ± 0.10 ?g/g) and opportunistically (0.812 ± 0.87 ?g/g). All individuals of our study population were denning and foraging in overgrazed cattle pastures. We used general linear models to predict glucocorticoid levels in relation to proximity to ranch quarters, and the rate of vigilance behavior directed towards cattle, domestic dogs, trains, and ranch activities. Preliminary analyses suggest that glucocorticoid metabolite profiles reflect the frequency of vigilance behaviors. Finally, our results indicated that the hoary fox was an ideal model species for advancing our understanding of the impacts of human activities on canid communities.



Abstract #84

Influence of external factors on hair cortisol concentrations

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Topic: Stress

Presentation type: Oral

Abstract:

Measuring hair cortisol has attracted interest as a long term parameter for chronic stress, but different studies support the hypothesis that locally produced cortisol affects levels in the hair. In an animal model the influence of different external factors (extensive brushing, application of a hyperemising fluid or a synthetic glucocorticoid) on the local cortisol production of hair were evaluated. Therefore eight sheep were sheared and the skin surface of the back was quartered, with three quarters being daily subjected to an external factor and one quarter remaining untreated (control). During the experiment daily fecal samples were also collected as a parameter of systemic cortisol concentrations. The fields were sheared again after three weeks and cortisol concentrations of all wool samples were determined by an enzyme immunoassay (antibody against cortisol-3-CMO:BSA) following extraction with methanol. Faecal cortisol metabolites (11,17-dioxoandrostanones) declined during the first few days, leading to significantly ($p < 0.001$) lower concentrations in weeks 2 and 3 (when compared with week 1). We found no significant difference in hair cortisol concentrations of the fields before treatment. After three weeks a significant difference could only be seen between the dexamethasone treated field (higher levels) and the control field ($p = 0.019$). Comparing the matched fields before and after treatment, the quarters exposed to extensive brushing and dexamethasone had higher hair cortisol levels ($p = 0.016$ and $p = 0.00987$, respectively). There was no significant difference at the field that was treated with hyperemising fluid and the untreated field ($p = 0.109$ and $p = 0.097$, respectively). We therefore advise caution when measuring hair cortisol concentrations as local external factors may impose a significant influence on the results.



Abstract #66

The impact of social dynamics and seasonality on changes in excreted testosterone and cortisol in an all-male colony of Malayan flying foxes

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

Malayan flying foxes (*Pteropus vampyrus*) are a seasonally breeding, socially complex species of megabat that naturally live in large colonies and exhibit peak activity at dusk and dawn. Breeding groups typically have a harem social structure. In zoo-managed *P. vampyrus*, surplus males are socially housed in single-sex groups, and management of agonistic social behaviors may be desired. This study validated enzyme immunoassays (antisera R156/7; R4866; C. Munro, UC Davis, CA) for fecal and urine testosterone and cortisol measurements in an all-male group of *P. vampyrus*. A total of 188 fecal and 231 urine AM samples were collected from 13 males aged 9-18 years old, between October 2014 to March 2015. Fecal and urine testosterone and cortisol were highly correlated (all $r^2 > 0.9$), thus subsequent analyses focused on urine hormone data. Behavioral observations collected during the study period were used to validate the hormone data. All occurrences of male-male agonistic interactions were recorded during a 1-2 hour observation period, 5 days/week.

Average monthly urinary testosterone was 20.48 ± 13.03 times higher during the breeding season (October) than baseline (December; $n = 8$; $p < 0.0001$). Cortisol was also elevated concomitant with seasonally-elevated testosterone ($r^2 = 0.98$). In the non-breeding season, individual differences in patterns of testosterone and cortisol were observed. A MANOVA analysis incorporating rates of behaviors with respect to urinary steroid concentrations revealed several important socially and seasonally complex interactions. Males with high rates of aggression exhibited elevated urinary testosterone ($p < 0.05$), whereas males that were the recipient of agonistic interactions exhibited increased cortisol ($p < 0.05$).

These results indicate that urine and fecal samples can be used as a non-invasive method to assess testosterone and cortisol metabolites in male *P. vampyrus*. Future research opportunities include investigation of effectiveness of gonadotropin-releasing hormone agonists to reduce testosterone and unwanted agonistic social dynamics.



Abstract #113

Male mountain gorillas' dominance hierarchies are not glucocorticoid or androgen mediated

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Topic: Misc

Presentation type: Oral

Abstract:

Androgen and glucocorticoid measurement provide physiological information on consequences of social and environmental stressors, which are proximate mechanisms underlying dominance hierarchies and timing of maturation. Their relationship to dominance and age is well documented in many primate species, with considerable variation in direction and magnitude of correlations. Despite the long research history on the mountain gorilla (*Gorilla beringei*) population in Volcanoes National Park, Rwanda, only one publication exists on the relationship among glucocorticoids, androgens, and male gorillas' age and rank. Since the previous study, the gorillas underwent marked social grouping changes, allowing reassessment of endocrine correlates of male age and rank in a different social environment with a bigger sample (mean=30.2 samples/male, range=8-65, n=733). We measured fecal glucocorticoid metabolites (FGM) and androgen metabolites (FAM) in three gorilla groups containing between six and eight males 10+ years old, plus a solitary male. Feces were extracted using a field method and analyzed at the Lincoln Park Zoo. FGM was quantified using a cortisol enzyme immunoassay (EIA; R4866; provided by C. Munro, University of California, Davis), and FAM via a testosterone EIA (R156/7; provided by C. Munro). As determined previously using urinary metabolites, there were no differences in FGM and FAM concentration in maturing (10-13 year old) and fully adult males (14+ years; $p=0.18$ & $p=0.24$). There was also no relationship between FAM and rank, but there was a trend ($p=0.09$) for subordinate males' FGM to be lower than dominant males'. The solitary male did not have different FAM or FGM concentrations than males in social groups ($p=0.14$, $p=0.25$). Unlike for many primate species in multimale groups, mountain gorillas' strong male dominance hierarchies do not appear to be mediated by androgens or glucocorticoids. This may be related to their apparent evolutionary history of single male groups, with current configurations representing a novel social environment.



Abstract #96

Boar urine pheromone exposure modifies estrous cycle length and regularity in same-sex housed female Red River Hogs
(*Potamochoerus porcus*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

Captive North American Red River Hogs (RRH) (*Potamochoerus porcus*) have variable reproductive success of unknown etiology. Determination of contributing factors to poor captive breeding would guide the development of management recommendations to optimize reproductive success. We hypothesized that estrous cycle irregularity occurs in RRH harems and that boar urine pheromones would normalize cyclicity. To test our hypothesis, fecal progesterone metabolite concentrations preceding and following pheromone introduction were assessed from a RRH harem (n=3 females) at Birmingham Zoo. Fecal samples were collected 2-3 times per week (Jan.-Sept. 2013). Females were exposed daily to RRH boar urine late June-early Aug. 2013. Fecals were processed by drying, sifting and weighing 0.2 grams of purified sample, after which they were extracted with 95% ethanol, dried under compressed air, and reconstituted in methanol. Progesterone (P4) metabolite concentrations were assessed via a P4 polyclonal enzyme immunoassay (R4859 antibody) using a 1:3000 sample dilution. P4 concentrations were analyzed with an iterative approach. The luteal phase of the cycle was characterized by at least three consecutive P4 points elevated two standard deviations above baseline and flanked on both sides by one or more points below baseline. Cycles preceding pheromone introduction were variable in length (n=7 cycles from 3 females over ~4 months; Range: 22-55 days) and not contiguous. Exposure to RRH boar urine induced all female RRH to cycle more regularly (n=6 cycles from 3 females over ~2 months; Range: 29-38 days). Average cycle length preceding pheromone exposure tended to be longer than average cycle length following pheromone exposure (preceding: 40.1 ± 4.4 days; following: 31.7 ± 1.5 days; $p=0.06$). In conclusion, irregular cyclicity may contribute to variable reproductive success in RRH, but females may be induced to cycle regularly when exposed to male urine pheromones. These findings suggest a strategy for the manipulation of breeding in captive RRH.



Abstract #74

Faecal epiandrosterone (epi-A) as an indicator of gonadal activity in spotted hyenas (*Crocuta crocuta*)

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Topic: Methodologies

Presentation type: Oral

Abstract:

The development of enzyme immunoassays (EIAs) to monitor faecal testosterone metabolites (FTM) constitutes a powerful tool to assess an animal's gonadal status non-invasively. However, steroid metabolism and excretion may differ significantly between species; non-invasive methods must be rigorously validated for each species before application. Validation requires both methodological and biological confirmation that the assay measures the target hormone or its metabolites and is sufficiently sensitive to detect changes in their levels in faeces as a function of well-defined experimental changes in physiological responses of the organism. Nevertheless, non-invasive studies are often done without both validation procedures because of their technically demanding and time consuming nature. A key aspect in the development of reliable androgen EIAs is to investigate and exclude possible cross-reactivities with structurally similar androgenic hormone metabolites not derived from androgen metabolism.

Our study describes an 'in-house' epiandrosterone EIA, based on epiandrosterone-3-HS-BSA (antibody) and epiandrosterone-3-HS-peroxidase (label), for the non-invasive monitoring of testosterone in a female dominated species, the spotted hyena (*Crocuta crocuta*). To our knowledge we present the first study combining three obligatory validation procedures. First, methodological validation was performed by running radiometabolism studies in both sexes followed by HPLC analyses in fecal extracts of captive as well as free-ranging spotted hyenas. Secondly, biological validation was performed by detecting an artificially induced increase in testosterone metabolite concentrations by a stimulation experiment and by demonstrating changes in FTM in relation to reproductive condition. Thirdly, we could exclude possible cross-reactivities of our EIA with unknown faecal glucocorticoid metabolites by demonstrating no responsiveness to ACTH. The development and validation of an epi-A EIA to measure FTM in spotted hyenas is an important breakthrough and will permit the assessment of variation in concentrations of this physiologically important hormone in wild spotted hyenas.



Abstract #57

Techniques for determining hormone concentrations in Southern stingray (*Dasyatis americana*) plasma using enzyme immunoassay

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Topic: Methodologies

Presentation type: Oral

Abstract:

The focus on maintaining sustainable captive populations in aquariums has led to a need for reproducible ways to monitor reproductive hormones in aquatic species. The southern stingray (*Dasyatis americana*) is currently being studied because of the prevalence of reproductive disease in captive females, however initial studies showed that plasma steroid hormone analysis using EIA was ineffective for measuring circulating steroid concentrations due to interference from protein matrices present in the blood, especially in females. Because EIA is an affordable and more ecofriendly option than RIAs, three treatments were investigated to develop EIA methodology for stingray plasma: 1) untreated, 2) treated with modified dissociation reagent (Arbor Assays), or 3) diethyl ether extracted. Treated and untreated samples were analyzed for progesterone (R4859) and estradiol (R0008) in female rays, and testosterone (R156/7) in male and female rays. Ether extracted samples and samples treated with dissociation reagent showed parallelism with the standard curve for all hormones whereas untreated samples only showed parallelism for testosterone and progesterone in females. Higher recoveries were obtained for plasma treated with dissociation reagent vs untreated or ether extraction, with recoveries of 94% (R=0.999) for progesterone, 91% (R=0.997) for estradiol and 99% (R=0.998) for male and 108% (R=0.999) for female testosterone, vs 19% (R=0.990)/ 80% (R=0.999) progesterone, 4% (R=0.992)/ 84% (R=0.999) estradiol, 2% (R=0.439)/ 63% (R=0.999) testosterone male and 9% (R=0.977)/ 71% (R=0.999) testosterone female for untreated or ether extracted respectively. Best results were obtained when plasma was treated with dissociation reagent. It is recognized that these values are measuring total circulating reproductive hormone concentrations rather than purported biologically available concentrations. Nonetheless, these results describe the first enzyme linked immunoassays available for stingray species that will allow aquariums to monitor the reproductive biology of this species.



Abstract #68

Is the serum androgen level during pregnancy related to sibling aggression in lynxes?

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Topic: Reproduction

Presentation type: Oral

Abstract:

Hormonal status of females during pregnancy may affect their maternal behavior after parturition and offspring characteristics. Among Felidae genera Lynx is famous for sibling aggression, which affects significantly cubs' development and in some cases may be fatal. We hypothesized that lynxes had higher androgens level during the pregnancy in comparison to other cat species that may be a reason of sibling aggression in lynx litters. The study was conducted at the biological station Tchernogolovka (50 km of Moscow). Data for blood samples were analyzed for three cat species: Eurasian lynx (*Lynx lynx*), Far-east wildcat (*Prionailurus bengalensis euptilura*) and house cat (*Felis catus*). The concentration of testosterone (Immunotekh, Russia, IF02-02) and androstenedione (DRG, USA, EIA3265) was measured by EIA in blood serum of adult males, adult non-pregnant and pregnant (15 days after mating) females. Blood samples were collected with anesthesia in lynxes and without it in two other species. In all three cats species concentration of testosterone has increased during the pregnancy. In average the level of testosterone increased about 75% in Eurasian lynx ($p < 0,05$), 122% in Far-East wildcat ($p < 0,05$), 57% in house cats ($p > 0,05$). In opposite to two other species where T level in pregnant females was lower than in males ($p < 0,05$), in Eurasian lynx it was similar in pregnant females and adult males. Pregnant lynx females with higher T level showed lower level of maternal care in the first month after parturition. Sibling aggression was described for the lynx litters with lower level of maternal care (allogrooming). Such way, androgens level in pregnant lynx females may be related with the phenomenon of sibling aggression, but there is no evidence that higher T level in other cats during pregnancy may affect offspring aggressiveness. This study was supported by RFBR 13-04-01465.



Abstract #43

Reproductive and stress monitoring in captive Asian elephants (*Elephas maximus*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

Of late, improper management of elephants in captivity has led to infertility and are thus facing demographic extinction. Endocrine profiling of reproductive hormones (progesterone and testosterone) and the stress hormone (cortisol) could facilitate better management and breeding strategies. We investigated reproductive and stress physiology of 35 captive Asian elephants for 6 to 27 months using a non-invasive method based on steroid analysis of 2700 dung samples collected 2-3 days/ week from 6 captive elephant facilities in south India. Faecal progestogen metabolites levels were measured using a progesterone monoclonal antibody (CL 425). Faecal glucocorticoid and androgen metabolites were measured using polyclonal antibody (R4866 and R156/7 respectively). All the antibodies were provided by Dr. Coralie Munro, University of California, Davis. Cycling was considered as based on the faecal progestogen and calculated as number of days from first peak until the next peak rise (Brown et al 1999). Non-cycling elephants were categorised based on lack of clear faecal progestogen cycling profile for more than six months. Based on the faecal progestogen levels it was deduced that the average length of anestrus cycle was 16 weeks (range 14–17 weeks) with a follicular phase of 6 weeks (range 5–7 weeks) and luteal phases of 10 weeks (range 9–12 weeks), respectively. About 70% of female elephants were cycling regularly and the remaining were either irregularly cycling or not cycling at all. Musth males showed increased faecal testosterone concentrations and exhibited increase in the faecal stress hormone. Elephants used in public festivals, processions and tourism activities showed increased 2 to 10 folds of faecal stress hormone from base level. The results indicate that captive elephants require periodic health care, better husbandry practices and scientific management for sustainable captive population in India.



Abstract #41

A gibbon of a different color: causes and consequences of color change in female white-cheeked gibbons (*Nomascus leucogenys*)

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Topic: Reproduction

Presentation type: Oral

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 8 years). While the triggers of color change have not been formally described, the assumption is that it is hormonally-mediated. Here, I report preliminary results on a study of hormones, behavior and color-change in female white-cheeked gibbons (*N. leucogenys*). Fecal samples were collected for three months from four female gibbons between the ages of 4 and 9 years. In addition to daily fecal samples, zoos provided monthly photos of females, and recorded occurrence of sexual behavior using a one-zero method. Zoos were asked to note on a calendar any days on which females were observed soliciting any males in the family group. Fecal samples were extracted and assayed for fecal progestagens using Arbor Assays™ Progesterone Immunoassay kit, which utilizes a mouse monoclonal antibody. Mean progestagen values were calculated for each female; elevation of 1.5 SD above the mean for at least three consecutive days was used as an indicator of elevation above baseline. Cycle length was calculated as the interval between the start of two consecutive luteal phases. Mean progestagen concentration (+SD) varied considerably, from 14.62+7.76 ng P/g feces in the youngest female to 44.32+26.01 in the oldest female. Cycle lengths varied from 22 to 27 days; no indication of cyclicity was detected in females under the age of four years. No sexual behavior was observed during the course of the study. Anecdotal evidence suggests that pelage color may influence the relationship between males and females, and females who are not fully blonde may risk being targets of aggression by prospective mates. Ongoing research will further elucidate patterns.



Abstract #58

A global pattern of female biased sea turtle sex ratios: feminization due to climate change?

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Topic: Misc

Presentation type: Oral

Abstract:

In order to conserve endangered sea turtles, sex ratio and sex-specific survival rate data in foraging sites have been underscored as key information that is currently lacking. Sea turtles do not have sex chromosomes; instead, sex is determined by incubation temperature of the egg during embryonic development (warmer temperatures produce females). This strategy, known as temperature-dependent sex determination, is sensitive to changing climate temperatures, potentially leading to large changes in population demography. Physical sex differences are not perceivable until sexual maturity, making visual identification of sex in immature turtles impossible. The two main techniques employed to determine sea turtle sex are laparoscopy or testosterone quantification. Laparoscopy is the ideal method for unequivocal sex determination, but requires invasive surgery. The collection of blood for testosterone measurement is less invasive and, therefore, more commonly used. Despite its potential, measuring testosterone (male turtles have distinctively higher testosterone concentrations) to determine sex has been applied at surprisingly few sea turtle foraging locations. To enhance sea turtle sex ratio studies, an ENZO Life Sciences testosterone ELISA was validated for six turtle species and application of the ELISA was comparable to an RIA that is traditionally used. Sex ratios were determined via hormone assay (and laparoscopy, n=5) at 5 green, 2 leatherback, 3 loggerhead, 2 Kemp's ridley, 1 olive ridley, and 1 hawksbill turtle foraging location/s; all were female biased. For example, we determined the first sex ratio for a threatened East Pacific green turtle foraging aggregation and found a 2.83F:1M ratio among all turtles; for putative immature turtles it was 3.5F:1M. Adult and immature male samples contained high testosterone (112.4–112,094.2 pg/mL) whereas immature and putative adult females had low concentrations (4.1–281.2 pg/mL). Sex ratio data provides important baseline information for investigating the potential effects of climate change and consequential feminization of sea turtle populations.



Abstract #88

Validation of a novel collection device for non-invasive urine sampling from free-ranging animals

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Topic: Methodologies

Presentation type: Oral

Abstract:

Recent advance in non-invasively collected samples have opened up new and exciting opportunities for wildlife research. Certain physiological markers can only be reliably measured from urine. Sufficient recovery of urine is still difficult for wildlife biologists, particularly for terrestrial and small bodied animals. We tested three collection devices (two saliva swabs, Salivette synthetic and Salivette cotton, and cotton first aid swabs) to permit urine collection of small volume from the ground. We collected urine samples from captive (n= 5) and wild macaques (n= 16) as well as humans (n= 4), measured volume recovery and analyzed concentrates of selected physiological markers (creatinine, C-peptide, and neopterin). Results were compared to a control (same sample not put through a collection device). Our results show that amongst our tested devices, the Salivette syntetic swabs was superior to the two alternative devices. Concentrations of creatinine, absolute C-peptide, C-peptide per creatinine, absolute neopterin, and neopterin per creatinine measured in samples collected with this device did not differ significantly from the control (Wilcoxon tests) and were also strongly correlated to it ($r= 0.93-0.99$ $p < 0.001$). Fluid recovery was also best for this device, at 84.5% of original volume using a hand centrifuge. The least suitable device is the First aid collection device: while absolute C-peptide and C-peptide per creatinine concentrations did not differ significantly from the control, creatinine concentrations were significantly lower than the control. In addition, these concentrations were either not or weakly correlated to the control. The Salivette cotton device provided intermediate results. Our results suggest that Salivette synthetic swabs are useful for the collection of small amounts of urine from the ground destined for assessing physiological parameters. They thus provide new opportunistic for field studies to incorporate physiological markers, particularly on smaller bodied and terrestrial animals and where urine collection is difficult



Abstract #67

Longitudinal progesterone and cortisol profiles recovered from baleen of North Atlantic right whales (*Eubalaena glacialis*)

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Topic: Methodologies

Presentation type: Oral

Abstract:

The baleen of mysticete whales consists of a series of "plates" on each side of the mouth, each plate containing long hairs embedded in a hard matrix. Plates grow continuously downward from the whale's upper jaw, wearing away at the lower end. In adult North Atlantic right whales (*Eubalaena glacialis*, NARW), a full-length baleen plate represents approximately 9-10 years of continuous baleen growth. We have recently demonstrated that baleen of bowhead whales contains detectable steroid hormones. The objective of this study was to determine whether baleen hormones in NARW contain an accurate retrospective record of reproductive history of the previous decade. We tested baleen from two individual NARW, using plates collected from stranded specimens. Both NARW were females with extensive sightings records and with known calving histories. Baleen subsamples were taken at 4cm intervals (each interval representing approximately 60 days of baleen growth) along the complete length of a 2m-long plate from each whale. A methanol extraction method was used to recover steroids from pulverized baleen powder. Progesterone and cortisol were quantified with Arbor Assays EIAs (kits K025-H1 and K003-H1, respectively). In both animals, progesterone and cortisol were detected at all locations along the baleen plate, including the distal tip (oldest baleen, exposed to seawater for approximately ten years). High-progesterone regions were clearly detectable in certain areas, and in all cases the estimated year-of-growth of these high-progesterone regions corresponded to the females' known pregnancies. This method may be suitable for determining intercalving intervals from baleen recovered from stranded specimens and potentially from historic archived baleen samples as well.



Abstract #104

Validating the use of skin swabs as a non-invasive technique to analyze stress hormones in various amphibian species

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Topic: Methodologies

Presentation type: Oral

Abstract:

Amphibians have been declining in both diversity and abundance due to habitat degradation and the prevalence of emerging diseases. Our goal was to investigate disease risk factors, including the effects of environmental changes. To evaluate amphibian stress physiology, we used skin swabs. For validation, we swabbed, using cotton-tipped applicators (Medline Industries), five amphibian species (n=2 per species) housed at Lincoln Park Zoo and Northern Illinois University including the American toad (*Anaxyrus americanus*), axolotl (*Ambystoma mexicanum*), red-spotted newt (*Notophthalmus viridescens*), green tree frog (GTF; *Hyla cinerea*) and northern leopard frog (NLF; *Lithobates pipiens*). Baseline swabs were taken and then individuals were held for 5 min to act as an acute stressor. Swabs were taken 0, 15, 30, 45, 60, 90 and 120min post-stressor. Swabs were placed in 1 ml of 70% ethanol until processing. Samples were mixed for 5 min and 500 µl was taken and dried (forced air at 60°C), reconstituted in 500 µl of PBS, sonicated 20 min and mixed for 30 min. Samples were analyzed using a cortisol EIA (R4866; C. Munro, University of California, Davis). For toads, baselines were 400 and 584 pg/ml and peaks were 2317pg/ml (at 45min) and 1577 pg/ml (at 90min), respectively. For axolotls, baselines were 748 and 631 pg/ml and peaks were 1025 pg/ml (at 90min) and 1076 pg/ml (at 60min), respectively. For newts, baselines were 629 and 458 pg/ml and peak was 1464 pg/ml (at 0min) and the other did not respond. For GTFs, baselines were 441 and 535 pg/ml and peaks were 870 (at 30min) and 1036 pg/ml (at 0min), respectively. For NLFs, baselines were 618 and 526 pg/ml and peak was 1011 pg/ml (at 15min) and the other did not respond. This novel method can be used to increase our understanding of the role of environmental changes on amphibian health.



Abstract #71

Testosterone levels detected in interscapular feathers of Eurasian sparrowhawk and Common buzzard in a non-breeding period

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

Testosterone (T) is described to be the most important steroid hormone mediator of sex differences. This hormone regulates reproductive processes and is involved in many physiological aspects such as regulating metabolic rate, immune system or feather growth in bird species. Quantifying this hormone over long periods of time is of paramount significance. However, T in birds has been mainly measured in blood samples. Feathers have been described as the unique method to represent long-term hormone circulating levels as they accumulate hormones during the moulting period. The objective was to determine feather T levels in raptors and test whether sex and age influenced its concentrations. Interscapular feathers were sampled as they moult after the breeding season. Eighteen Eurasian sparrowhawk (*Accipiter nisus*) and fifteen Common buzzard (*Buteo buteo*) admitted at Torreferrussa Rehabilitation Center (Spain) were analyzed. Physiological data such as age (young, subadult, adult) and sex were recorded. A methanol-based extraction technique and a competitive enzyme immunoassay kit (Ref. 402510; Neogen® Europe, Ayr, UK) were used for feather T determination. Validation tests were performed with successful results. The intra-assay coefficient of variation obtained was 7.6%, the mean error percentage was $22.2 \pm 23.5\%$ with an R² of 94% and the average recovery percentage was $105.9 \pm 6.6\%$. The T mean concentration was 50.4 ± 10.1 and 47.5 ± 11.5 (pg T/mm feather) for Eurasian sparrowhawk and Common buzzard, respectively, without statistical differences between species ($p=0.2$). In Eurasian sparrowhawk, neither age ($p=0.8$) nor sex ($p=0.3$) showed differences in T levels. In Common buzzard, no age differences were found ($p=0.6$). However, in this species males showed significantly higher T levels than females ($p<0.01$). For first time, long-term T levels are achieved in raptor species using interscapular feathers. Data found indicates a different influence of sex on T concentration depending on species and suggests caution considering sex as a physiological source of variation.



Abstract #102

Developing the technique to measure steroid and thyroid hormones in "blow" (respiratory vapor) of large whales

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Topic: Methodologies

Presentation type: Oral

Abstract:

Until now, it has been difficult to collect physiological samples for hormone assessment from free-swimming whales. A major challenge is that the sample types commonly used for hormone analysis (e.g., blood, feces) are logistically impossible to collect from large whales at sea. The novel approach of sampling respiratory vapor ('blow') has shown considerable promise for whales, but important methodological issues still need to be addressed. This study evaluated several technical factors that may play a crucial role in the successful quantification of blow hormone values. We performed both laboratory experiments and field trials for three different sampling materials that show promise in the collection of blow (veil nylon, nitex nylon and polystyrene plate), focusing on a well-catalogued whale species, the North Atlantic right whale (*Eubalaena glacialis*). We used commercially available enzymeimmunoassays (Arbor Assays) to measure five steroid hormones (testosterone, #K032-H1; progesterone, #K025-H1; estradiol, #K036-H1; cortisol, #K003-H1) and aldosterone (#052-H1) and a radioimmunoassay to measure thyroid hormone (triiodothyronine; #06B-254215, MP Biomedicals). Experimental results ($n = 128$ samples) showed that recovered concentrations (low 0.1 ng/mL, medium 1.0 ng/mL and high 10 ng/mL) of known hormones (prepared as mock blow solutions) matched expected ratios, regardless of sampler type (all $P > 0.05$). However, progesterone recovery was significantly improved by 81% with an ethanol rinse ($P < 0.001$). Our results demonstrate the reliability of field sample storage; efficiency of hormone extraction methods; and assessed potential assay interference from sampling materials. Most significantly, blow samples collected from NARW ($n = 100$ samples from known whales) were analyzed to tackle the issue of variable water content in samples. Results showed that urea (a potential volume adjuster) was detectable in blow samples. Our findings will provide the critical groundwork for defining reliable sample preparation and analysis techniques to ensure data quality for measuring hormones in whale blow.



Abstract #97

High Non-specific Binding or Matrix Interference in Enzyme Immunoassays Used for the Measurement of Reproductive Hormones in Cetacean Blow

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Topic: Methodologies

Presentation type: Oral

Abstract:

Blow hormone concentrations using enzyme immunoassays (EIAs) have been reported in the North Atlantic right whale and the beluga. Our objective was to examine the utility of the aforementioned method in the beluga for measuring testosterone, progesterone and progestagen concentrations in blow from killer whales, bottlenose dolphins and beluga. Samples were collected onto nylon mesh (Elko Filtering, Miami, FL) pre-treated with 70% ethanol. After collection, mesh was placed in a 50ml conical tube then centrifuged to allow for collection of sample from the tube bottom. Potential residual sample in the mesh was extracted using 70% ethanol. Dried extracts were reconstituted in assay buffer then analyzed with the centrifuged neat sample using in-house (progestagen [CL425], testosterone [R156/7], University of California, Davis, CA) or commercial (progesterone [#582601], Cayman Chemicals, Ann Arbor, MI) EIAs. To check for potential matrix interference (MI) from collection materials or non-specific binding (NSB), water samples from enclosures and Milli-Q water (placed onto collection materials and processed identically) were tested. In all assays we found a high degree of NSB or MI for both water sample types placed on nylon mesh (range: 58.2±3.3% to 74.7±1.2%) compared to neat water (range: 91.03±3.3% to 106.6±3.4%). An alternative collection method using an untreated 0.2µm polyethersulfone filtration unit (ThermoScientific, Waltham, MA) was examined. Percent binding was within acceptable limits (range: 87.8±0.9% to 100.5±2.3 %). Extracted water samples had high NSB or MI, regardless of the collection apparatus used (range: 17.2±0.3% to 57.0±0.6%) Due to the high NSB or MI observed of samples collected from nylon mesh with or without extraction, this collection method is not considered an accurate means of measuring hormone concentrations in cetacean blow under our laboratory conditions. These findings warrant further examination of blow collection using a polyethersulfone filter unit, if the apparatus can be modified for practical use in zoo and field-settings.



Abstract #70

Metabolism of PGF2alpha in the Eurasian lynx (*Lynx lynx*) and the leopard cat (*Prionailurus bengalensis euptilura*)

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Topic: Methodologies

Presentation type: Oral

Abstract:

Methods for monitoring endocrine activities are useful tools for reproduction management. In particular, successful captive breeding of endangered felid species requires reliable methods for pregnancy diagnosis. Fecal PGFM (PGF2alpha metabolite) has been confirmed as an appropriate indicator for pregnancy in several feline species. Surprisingly, peak levels of PGFM secretion differed dramatically between species and it was believed that prostaglandin composition and metabolism pathways may be different as well. In addition, a study on fecal PGFM metabolites in Iberian lynx revealed that only a minor proportion of immunoreactivity was PGFM; whereas major immunoreactive peaks could not be identified based on co-elution with commercial prostaglandin standards and LCMS analyses. Thus, their chemical structure and origin remained unknown. To investigate the metabolism of PGF2alpha, we carried out a radiometabolism study with two Eurasian lynxes and two leopard cats. Each individual received an i. m. injection of 100 and 25 μ Ci tritiated PGF2alpha, respectively. HPLC separations of fecal metabolites were carried out to identify radiolabelled and immunoreactive metabolites.

Our results confirmed that radiolabelled fecal metabolite compositions differ between the two feline species. In addition, individual differences were obtained. Coincident results were obtained regarding the characteristics of radiolabelled metabolites demonstrating only very low proportions of PGFM in both species. In the Eurasian lynxes and the leopard cats the major proportions consisted of two unknown metabolites with a polarity similar to PGF2alpha. In lynxes, two additional metabolites were obtained with a polarity similar to tetranor-PGFM. All radiolabelled metabolites were recognized by our PGFM antibody, which did not express any cross-reactivity to both, PGF2alpha and tetranor-PGFM. Based on the metabolite pattern we hypothesize that PGF2alpha is further metabolized to more polar dinor- and tetranor metabolites. Current research focuses on their identification aimed to use these metabolites to improve pregnancy detection in felids and probably other carnivores.



Abstract #94

What makes my nest best? The effect of captive environment on stress and reproduction in the captive whooping cranes

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Topic: Reproduction

Presentation type: Oral

Abstract:

Habitat design is constantly evolving and many factors must be considered. This study investigated the effects that having a ponded enclosure has on reproductive output and stress of captive whooping cranes (*Grus americana*). We examined fecal hormone metabolites of 8 adult whooping crane pairs housed at USGS-Patuxent Wildlife Research Center. During the first study year, all individuals were housed in traditional dry pens. In year two, prior to breeding season, all pairs were moved to new pens, 4 to similar dry pens (control group) and 4 to ponded pens (treatment group). All analyses were tested using linear mixed effect models. Glucocorticoid means (in ng/g feces) were higher in year 1 compared to year 2 in the treatment group of both sexes (male: $p=0.0091$; 890.44 ± 117.12 vs. 771.87 ± 265.78 ; female: $p=0.0001$; 1004.55 ± 395.73 vs. 780.28 ± 227.82). Glucocorticoid means were not lower in the control group of either sex (male: 901.38 ± 162.88 vs. 830.37 ± 265.79 ; female: 1064.52 ± 355.99 vs. 1151.21 ± 480.21). Additionally, estrogen means were higher (control: $p=0.0215$; 218.39 ± 140.93 vs. 282.18 ± 190.64 ; treatment: $p=0.0003$; 282.18 ± 135.29 vs. 295.18 ± 205.22) while progesterone means were lower (control: $p=0.0003$; 81.49 ± 28.32 vs. 64.89 ± 18.53 ; treatment: $p=0.0001$; 215.56 ± 160.68 vs. 68.43 ± 21.04) in year 2 compared to year 1 in the females of both groups. This change in reproductive hormones appears to be a result of individuals in both groups laying eggs in year 2 rather than solely the presence of the ponds. Moreover, a pair who had not laid an egg in the previous 3 yrs when moved to a ponded pen laid 2 fertile eggs in year 2. Anecdotal behavior observations were made; previously passive pairs, when moved to ponded pens, began defending territories and were aggressive towards keepers. Overall we saw a positive effect when a pond is included in the pens and these preliminary results indicate the importance of appropriate exhibit environment for animals housed in captivity.



Abstract #73

Measurement of cortisol concentrations in scales of a wild freshwater fish (*Squalius laietanus*)

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Topic: Stress

Presentation type: Oral

Abstract:

Methods for evaluation of steroid production in fish have been developed since few years ago, but they are still progressing in relation to animal management and thus animal welfare. Steroid hormones are able to diffuse from capillaries into the feather, hair and shed skin where they are deposited during its growth, providing an historical record of an individual hypothalamic–pituitary–adrenal axis activity. We hypothesized that fish scales are able to accumulate cortisol, the main glucocorticoid in fish. Thus, the aim of the present study was to evaluate if cortisol could be detected in scales of a wild freshwater fish by enzyme immunoassay (EIA). Four individuals of Catalan chub (*Squalius laietanus*) were caught in a stream in the Besòs basin as part of a research project of environmental health assessment. Blood, scales, branchial mucus and caudal fin samples were collected immediately after sacrifice. Scales and the caudal fin of each individual were washed with a 2% NaOH solution. Once dried and minced, a methanol-based technique was applied for hormone extraction. To concentrate the hormone extract, the solvent was evaporated and reconstituted with the assay buffer. Conversely, plasma and branchial mucus were previously diluted. Finally, cortisol was measured using a cortisol EIA kit (Salimetrics LLC, State College, PA). Cortisol concentrations (mean \pm SD) were 1.5 ± 0.3 ng cortisol/g in scales, 5.9 ± 3.6 ng cortisol/g in caudal fin, 430.3 ± 27.6 ng cortisol/ml in plasma and 119.4 ± 45.3 ng cortisol/ml in branchial mucus. A tendency for a positive correlation between scales and plasma cortisol concentrations was observed ($r = 0.911$, $P = 0.089$). Results show for the first time that cortisol can be measured in the scales of an endemic wild fish. This new matrix could be a potential tool for assessing hypothalamic–pituitary–interrenal axis in fish species.



Abstract #72

Monitoring long-term stress through hair cortisol analysis in wild chimpanzees (*Pan troglodytes*)

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Topic: Stress

Presentation type: Oral

Abstract:

Elevated long-term stress increases the susceptibility to infectious illnesses and reduces fertility, and therefore it can decrease animal fitness. To investigate anthropogenic influences on the stress level of wild chimpanzees (*Pan troglodytes*) we measured cortisol concentrations in hair (HCC) using LC-MS/MS which enabled an estimation of the integrated stress levels over three months. Hair samples were collected from nests of chimpanzee communities in Western Uganda with varying anthropogenic impact: severe human-wildlife conflicts ($N=14$), ecotourism (low tourist season: $N(LT)=31$, high tourist season: $N(HT)=19$), no human impact ($N(LT)=49$, $N(HT)=37$), and illegal logging and hunting ($N=45$). Earlier investigations on captive animals had shown systematic cortisol decrease along the hair shaft if chimpanzees were exposed to ambient weather conditions ($F(2,1)=72.4$, $p<0.001$, mean decrease per cm = $20\pm 25\%$). An ANCOVA with planned contrasts revealed that, similarly, the covariate 'nest age' had a significant effect on HCC ($F(2,187)=18.0$, $p<0.001$). While accounting for nest age, HCC revealed significant differences in stress levels between groups. As expected, HCC was significantly higher in chimpanzees experiencing severe human-wildlife conflicts than communities living in a nearby forest ($t(187)=2.3$, $p<0.05$). Furthermore, tourism chimpanzees exhibited comparable HCC in the low and high tourist season. But while there was no difference between the tourism group and the control group (adjacent community without human impact) in the low tourist season ($t(187)=1.5$, $p=0.13$), unexpectedly, tourism chimpanzees exhibited less HCC than the control group in the high tourist season ($t(187)=-4.0$, $p<0.001$). Although the underlying stressor affecting the control group in the high season is unknown due to the lack of knowledge about that group, our results suggest that tourism was not a major stressor for the tourism chimpanzees. In conclusion, hair cortisol analysis is a powerful tool that allows estimating the long-term stress level of wild chimpanzees regardless of the animals' habituation status.



Abstract #85

Fecal thyroid hormone measurements as a non-invasive method to monitor energy balance in yellow-breasted capuchins (*Sapajus xanthosternos*)

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Topic: Methodologies

Presentation type: Oral

Abstract:

Energetic condition is a key parameter contributing to individual fitness. However, the accurate monitoring of energetic condition in wild-living animals is a significant methodological challenge. Recently, measuring urinary C-peptide has proven to be a suitable method to non-invasively assess energy balance in wild-living animals. Because urine collection is not always feasible in the wild, it is essential to develop alternative methods to monitor energy balance. Thyroid hormones are involved in the regulation of metabolic processes and are therefore a candidate biomarker for the assessment of energy balance. During periods of low energetic intake, excretion of the thyroid hormone T3 in serum is reduced, leading to a decrease in metabolic activity. Previous studies suggest that energy balance can be monitored through measuring changes in fecal thyroid levels. We validated a commercial total T3 ELISA (Ref. RE55251, IBL International GmbH, Hamburg, Germany) to measure immunoreactive T3 (iT3) in feces of yellow-breasted capuchins. We restricted caloric intake of seven captive males, assessed daily group caloric intake, and determined individual iT3 levels. Chemical validation showed that the assay reliably measures iT3 levels in fecal samples of yellow-breasted capuchins in terms of parallelism, extraction efficiency (average recovery after spiking experiment: 75.3%–106.1%) and recovery (average recovery after spiking experiment: 86.12%–90.63%). Individual iT3 levels were significantly associated with daily group intake of calories; however, individual thyroid levels varied substantially resulting in overlap across individuals between feeding and restriction periods. Therefore, changes in energy balance should be assessed only through repeated measures within individuals and not between individuals. The results indicate that fecal iT3 can be reproducibly extracted and measured, and can serve as a biomarker to detect changes in energy balance of yellow-breasted capuchins. Importantly, measuring iT3 levels in feces might present a suitable alternative for monitoring energy balance when urine sample collection is impossible.



Abstract #82

Stability of Asian elephant's fecal glucocorticoid metabolites concentrations (fGCM) in a tropical environment.

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Topic: Methodologies

Presentation type: Oral

Abstract:

Time can be a critical factor when monitoring fecal glucocorticoid metabolites (fGCM) in wild Asian elephants (*Elephas maximus*) in the tropical rainforests of Malaysia. During fieldwork, it is necessary to wait until the elephant has moved to a safe distance before samples can be collected. To study the stability of wild Asian elephant's fGCM in field conditions, eighty fresh fecal piles were collected from eight female and two male elephants in National Elephant Conservation Center, Kuala Gandah. For each fecal pile, repeated subsamples were collected resulting in 690 subsamples grouped into the following categories: "fresh defecation", "below 2 hours (post-defecation)", "after 2 hours", "after 4 hours", "after 6 hours", "after 8 hours", "after 11 hours", "1 Day", "1.5 Days" and "2 Days". The fecal piles were also subjected to different environmental treatment, which included: (1) sun exposure – placed under canopy or open field and (2) water treatment – with or without approximately one liter of water poured over the fecal pile. Subsamples were extracted using a wet-weight extraction technique (Walker et al., 2002) and analyzed with a corticosterone enzymeimmunoassay (CJM006 by Coralie Munro, UC Davis) (Watson et al., 2013). After excluding outliers, data from 76 fecal piles and 685 subsamples were used for statistical analysis using a linear mixed model. The fGCM concentrations from samples up to eight hours old were comparable to fresh fecal samples ($p > 0.05$), after that, fGCM concentrations increased continuously before dropping to lower concentrations on the second day. In addition, fecal piles exposed to sun had higher fGCM concentration ($p > 0.05$). No differences were detected between fecal samples treated with water and those untreated ($p < 0.05$). With better understanding of environmental factors affecting fGCM, we will be able to develop better experimental designs for monitoring of free-ranging wildlife.



Abstract #63

Fecal cortisol metabolites in mandrills (*Mandrillus sphinx*) reintroduced to Conkouati-Douli National Park, Republic of Congo

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

In 2013-2015, the Jane Goodall Institute piloted a rerelease of wild-born mandrills confiscated in the Republic of Congo (RoC) into Conkouati-Douli National Park, RoC. After rehabilitation at Tchimpounga Chimpanzee Rehabilitation Center, animals were transferred to a pre-release enclosure at the release site for ~6mo and ultimately released into the park.

Stress is implicated as a major factor in animal reintroduction failures; thus, our objective was to monitor the welfare of release individuals as part of an integrative approach that included quantification of fecal glucocorticoid metabolites (FGM). We hypothesized that FGM would increase initially after animals were transferred to the pre-release site and the park and return to baseline levels as animals acclimated.

After evaluating a panel of FGM in zoo-managed mandrills for validation, we selected 11- β -hydroxyaetiocholanolone (69a) as a biomarker to monitor in release individuals during each release phase. Fecal samples (n=2800) were collected from known individuals, extracted in 90% ethanol, and dried (50°C). FGM were measured in a sample subset (n=150; representing the first 3 males released) by enzyme immunoassay (EIA) using antiserum 5- α -androstane-3 β ,11 β -di-ol-17-one-CMO:BSA (Ak3138/6/99).

Animals had comparable FGM ($p>0.05$) and had 1.75-2x higher FGM after transfer to the release site ($p<0.05$). FGM subsequently returned to pre-transfer concentrations (442 ± 34 ng/g) prior to animal release into the park. Interestingly, after release to the park, two males tended to have FGM comparable to those prior to transfer ($p>0.05$), while one male had sustained elevated FGM post-release concomitant with an arm injury and bite wound.

Based on FGM, the ~6mo acclimatization prior to release appeared to minimize stress levels associated with reintroduction. Our results will help inform IUCN guidelines for future primate reintroductions by providing an objective measure to assess how long animals should spend at each phase of a release to avoid compounding effects associated with the stress of reintroduction.



Abstract #48

Non-invasive monitoring of adrenocortical function as a measure of stress in captive, semi-captive, and free-ranging Golden Langurs (*Trachypitecus geei*)

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Topic: Stress

Presentation type: Oral

Abstract:

Probably evolved in geographic isolation, the endangered Golden langur can only be found in a small region of western Assam, India, and in the neighbouring area of Bhutan. Its overall declining population is threatened by human activity through habitat fragmentation and degradation. An area-wide monitoring program assessing the perception of environmental-related stressors could therefore help to identify highly afflicted areas. To provide a reliable non-invasive method for determining stress-related physiological responses, we examined the suitability of enzymeimmunoassays (EIAs) for monitoring adrenocortical function in Golden Langur based on faecal glucocorticoid metabolite (fGCM) analysis.

We performed an ACTH challenge (intramuscular injection of Acton Prolongatum, 6 IU/kg) on two 4-5 year old individuals kept in households in the Basugaon and Holtugaon area, Kokrajhar, India. Faeces were collected 1 day prior to 10 days following treatment, and resulting hormone extracts were measured for fGCM concentrations using five different EIAs from UVM Vienna, namely cortisol, corticosterone, 5 β -pregnane-3 α ,11 β ,21-triol-20-one (measuring 3 α ,11 β -diol-CM), and 11-oxoaetiocholanolone I & II (detecting 11,17 dioxoandrostanes and fGCMs with a 5 β -3 α -ol-11-one structure, respectively). The 11-oxoaetiocholanolone I EIA performed best resulting in a 130% (male) and 620% (female) increase in fGCM levels 48h post-injection. Subsequently 12 individuals at Chakrasila Wildlife Sanctuary (free-ranging), 5 animals at Umananda Island (semi-captive), and 3 males housed at Assam State Zoo cum Botanical Garden (captive) were monitored between 5-30 days, and a total of 227 faecal samples collected (one sample/individual/day). Significantly higher overall fGCM levels were found for the semi-captive group compared to the free-ranging population ($T_{20,118}=2031$; $P<0.001$), and also the 3 zoo-housed animals differed significantly in terms of their fGCM concentrations ($H_2=49.3$; $P<0.001$; post-hoc: AvsB, AvsC, BvsC; $P<0.01$).

The ability to reliably assess adrenocortical function in Golden langur now provides us with a practical non-invasive tool to monitor responses to stressors in this highly threatened, sacred species.



Abstract #62

Ecological variations impact the stress status of free-ranging Asian elephants.

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Topic: Stress

Presentation type: Oral

Abstract:

Ecological perturbations such as seasonality, resource availability and anthropogenic factors are known to influence stress status of free-ranging Asian elephants. However, how eco-variations influence animals' stress physiology is not assessed. We evaluated the influence of seasonality, animals' body condition, gender, age and habitat quality on the stress status of free-ranging Asian elephants in the Bandipur National Park (BNP) and the Nagarhole National Park (NNP), India. For this, we collected 250 fresh fecal samples of elephants during wet and dry seasons in 2013. We assessed the animals' body condition score (BCS); scale: 1 to 5; 1, emaciated and 5, obese conditions. Fecal glucocorticoid metabolites (fGM; $\mu\text{g/g}$) were measured (in Dr. Seshagiri's lab) using a group-specific 11-oxoetiocholanolone EIA (lab-code: 72T with an antibody against 11-oxoetiocholanolone-17-CMO:BSA and biotinylated-11-oxoetiocholanolone as a label). Our results show that while dry and wet seasons did not show any difference in fGM levels, individuals ($n = 42$) from BNP had a higher fGM level (1.12 ± 0.2) than those ($n = 42$) from NNP (0.67 ± 0.05 ; $p < 0.05$). BCS and fGM levels showed a significant negative correlation ($p < 0.05$) between score-1 (fGM: 0.94 ± 0.3) and score-3 (0.89 ± 0.2). Moreover, fGM levels were higher in females (1.09 ± 0.17 , $n = 50$) than in males (0.61 ± 0.08 , $n = 36$; $p < 0.05$), as well as higher in adults (0.89 ± 0.08 ; $n = 64$) than in sub-adults (0.46 ± 0.06 ; $n = 12$) or juveniles (0.40 ± 0.06 ; $n = 7$); both males and females were included. These results indicate that among the investigated parameters, BCS, habitat and age of animals influence the stress status of free-ranging Asian elephants. Our ongoing studies on a large sample size would provide a more detailed understanding of ecological factors affecting stress physiology of Asian elephants.



Abstract #55

The measurement of female reproductive steroid hormones in marsupial urine

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Topic: Reproduction

Presentation type: Oral

Abstract:

Although fecal progesterone metabolites have been successfully analyzed in the southern hairy-nosed wombat (SHNW), the measurement of estradiol metabolites has been problematic, preventing adequate characterization of estrus. The specific aim of this study was to develop and biologically validate a protocol for the measurement of SHNW urinary reproductive steroid hormones. Preliminary data was collected from five captive SHNW conditioned to provide urine samples on demand. Daily samples were collected fresh and stored frozen (-20°C) until enzyme-immunoassay hormone analysis of estrone-3-glucuronide (E1C) and progesterone (P) (R522-2 and CL425 antibodies respectively; C. J. Munro, UC Davis, USA) urinary metabolites. All samples were evaluated for creatinine (Cr) and specific gravity (SG). The mean baseline for E1C was 0.42 ± 0.10 ng/mg Cr (range 0.25 – 0.54) and the mean P baseline was 1.54 ± 0.57 ng/mg Cr (range 1.00 – 2.75). Changes in female reproductive behaviors, such as increased activity, fence line interaction with males, and in one case, homosexual behavior, coincided with periods of elevated E1C two standard deviations (SD) above baseline. For one confirmed pregnancy, P levels increased 2 SD above baseline (2.54 ng/mg Cr) after mating and peaked the day of parturition (7.026 ng/mg Cr), returning to baseline levels five days after birth. These results demonstrate a biological validation of both assays for SHNW urine. Additionally, there was a strong correlation between Cr and SG (r value = 0.80, P = 0.001), demonstrating a potential alternative urine concentration standardization method. This is the first study to detect and successfully analyze urinary reproductive steroid metabolites in SHNW. The measurement of urinary hormones should provide a more defined description of the SHNW reproductive cycle that can ultimately be applied to the captive reproductive management of the critically endangered northern hairy-nosed wombat.



Abstract #100

Understanding the role of neurohormones in hyperprolactinemia of African elephants (*Loxodonta africana*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

Many zoo elephants do not cycle normally and for Africans, it often is associated with hyperprolactinemia. No underlying cause or treatment for hyperprolactinemia has been identified for elephants, but the syndrome could negatively affect health and reproduction. In humans, dopamine agonists and serotonin antagonists have been used to successfully treat hyperprolactinemia. An investigation into neurohormone regulation in this species was therefore warranted.

Weekly urine and serum samples preserved with ascorbic acid were collected for 8 consecutive months to characterize neurohormone profiles from 10 elephants that have been diagnosed with hyperprolactinemia, along with 10 cycling females that served as normal controls (through two estrous cycles), and 10 non-cycling, low prolactin females that served as negative controls.

The EIAs for dopamine (Dopamine Research ELISATM BA E-5300, Rocky Mountain Diagnostics, Inc.), oxytocin (Oxytocin ELISA, ADI-901-153, Enzo Life Sciences) and serotonin (Serotonin Research ELISATM BA E-5900, Rocky Mountain Diagnostics, Inc.) were validated for use with African elephant serum and urine. The only exception was serum in the dopamine assay, where we saw no cross-reactivity. Therefore, dopamine was measured in urine only. A double antibody EIA method (CL425; C. Munro, UCDavis, CA) was utilized to determine urinary progesterone concentrations. Serum prolactin was measured by a heterologous RIA validated for elephants (Brown and Lehnhardt, 1997).

A negative correlation has been found between urinary dopamine and serum prolactin concentrations ($r = -0.425$, $p=0.01$), while positive correlations were found between urinary serotonin and serum prolactin concentrations ($r = 0.403$, $p=0.02$) and serum oxytocin and serum prolactin concentrations ($r = 0.672$, $p=0.02$). Thus, preliminary data indicate these immunoassays measure biologically relevant analytes, and that these neurohormones may function in prolactin control in elephants similar to that in other species.



Abstract #52

Anti-Müllerian hormone in female cheetahs (*Acinonyx jubatus*): Effects of age and deslorelin

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Topic: Reproduction

Presentation type: Oral

Abstract:

Serum anti-Müllerian hormone (AMH) concentrations have been shown to decline with age in female mammals, owing to the age-associated decline in the number of ovarian follicles. However, AMH concentrations show considerable variation among animals of the same age, and a relatively low AMH for a given age might indicate an increased risk for early onset subfertility and reproductive senescence. Because some cheetahs in zoos and conservation facilities reach a relatively advanced age before breeding, we have undertaken a study to determine if AMH testing can help to optimize the management of cheetah breeding programs. This is especially important when assisted reproductive technologies are being considered, because AMH has been shown to correlate with the ovarian response to gonadotropin stimulation. We measured serum AMH concentrations in 22 females ranging in age from 1.7 to 14.0 years (AMH range 0.08-1.95 ng/mL) after validating the Beckman Coulter Gen II ELISA for cheetahs (parallelism, intra-assay CV=3.9%). The inverse relationship between AMH and age was statistically significant ($r = -0.76$, $p < 0.001$). Six females were sampled at approximately 7 years of age, and their AMH values were highly variable (0.37-1.62 ng/mL). Six additional cheetahs had been treated with deslorelin 2-18 months prior to sample collection. Preliminary results suggest that long-term deslorelin treatment suppresses AMH concentrations. For example, one of the cheetahs was sampled 2 and 6 months after deslorelin administration, and her AMH declined from 2.56 to 0.49 ng/mL. Four other females that had been treated 8-18 months prior to sample collection had relatively low AMH concentrations for their ages. Collectively, our results show: 1. AMH declines with age in female cheetahs, 2. Inter-individual variation in AMH is relatively high at an age when cheetahs are at their reproductive prime (3-8 years), and 3. When used for contraception, deslorelin might suppress AMH concentrations.



Abstract #89

Endocrine monitoring of reproductive function in the African lesser bushbaby, *Galago moholi*

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Topic: Reproduction

Presentation type: Oral

Abstract:

Hormones play an important role in both male and female reproductive physiology and behaviour, and therefore endocrine correlates have been used to explain respective patterns, especially in numerous new world primates. However, studies linking reproductive hormones with key reproductive events are still comparatively scarce for African prosimian species, especially for *Galago moholi*, a small primate species distributed throughout southern Africa. In this study we characterized the endocrine patterns present during reproductive periods using seven captive mating pairs, while also monitoring free-ranging individuals from the surrounding area for 8 months at Ithumela Primate Sanctuary, South Africa. We collected faeces tri-weekly from our captive individuals as well as from free-ranging individuals whenever caught. In addition, we frequently observed the individuals and determined testis size from animals of both populations. Faecal steroid metabolite levels were determined by using antibodies against 20-oxopregnanes, 17β -oestradiol-17-HS, 5 α -Androstan-3 α -ol-17-on respectively. For males, our data confirms the presence of two equally important reproductive periods, both associated with an increase in faecal androgen metabolite concentrations as well as an elevation in testis volumes. For the females, we determined a follicular phase of 14.2 ± 1.04 (mean \pm SD) days and luteal phase of 19.12 ± 1.53 days, allowing us to successfully calculate the length of the ovarian cycle (33.5 ± 1.3 days). Monitored females became pregnant during the study period (May, n=4; September, n=1), displaying an increase in both oestrogen and progestagens metabolite concentrations, above individual hormone baseline level, until parturition ($128 \pm$ SD 3.3 days). Two of the monitored females did not conceive and displayed an irregular ovarian endocrine activity. The cross-sectional data gathered from the free-ranging *G. moholi* population confirm the endocrine results described above. This is the first study elucidating the reproductive endocrine patterns and their behavioural correlates, encompassing reproductive, non-reproductive periods and pregnancy periods, in the African lesser bushbaby.



Abstract #95

Fecal hormone metabolites and ultrasonic examination of gestation in giant anteater (*Myrmecophaga tridactyla*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

Reproductive hormone measurements in female giant anteater were conducted previously (Patzl et al., Anim Rep Sci, 1998; Knott et al., Rep Biol Endocrin, 2013). Gestation length is about 180 days. Knott et al. interpreted a sharp increase of progesterone metabolite levels by mid gestation as evidence for delayed implantation. However, there was no morphological evidence presented by Knott et al., to underline the statement of 'evidence for delayed implantation in the giant anteater'.

We conducted fecal hormone metabolite measurements, using a pregnandiol assay and an assay for total estrogens in n=6 pregnancies of six females. In parallel, we performed ultrasonic examinations for pregnancy diagnosis in weekly intervals, starting at about Day 60 of gestation. Fecal pregnane levels increased by mid gestation, whereas fecal estrogens increased during the last 2 months of gestation. Increasing hormone levels are most likely caused by placental hormone production. Peak concentrations in both hormones occurred during the last month of pregnancy. Pregnane levels were characterized by high individual variability, and are not recommended for pregnancy diagnosis. Estrogen measurements during the last two months of gestation would be more appropriate for this purpose. However, ultrasonic imaging techniques are the preferred method for pregnancy diagnosis. By day 70, the developing fetus is about 2 cm in size and its heartbeat is clearly visible. By mid-gestation (day 90) the length of the U-shaped fetus (nose to tip of tail) is already 8 - 10 cm, the size of the uterus is about 8 x 8 cm, as compared to 3 x 4 cm of the non-gravid uterus. Embryonic diapause at the blastocyst stage, as assumed to last until mid-gestation by Knott et al., (2013) would certainly not be visible by ultrasonic examinations. Thus our results clearly contradict the misleading suggestion of embryonic diapause occurring in the giant anteater.



Abstract #39

Reproductive health of female elephants in North American zoos and association of management with pituitary-ovarian problems

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

As part of a multi-institutional study of zoo elephant welfare in Association of Zoos and Aquariums zoos, epidemiological methods were used to determine what factors in the zoo environment are associated with reproductive problems, including ovarian acyclicity and hyperprolactinemia. Bi-weekly blood samples were collected from 75 Asian and 95 African elephant females (8 – 55 years of age) over a 12-month period for analysis of serum progestogens and prolactin. Females were categorized as normal cycling (regular 13- to 17-week cycles), irregular cycling (cycles longer or shorter than normal) or acyclic (baseline progestogens, <0.1 ng/ml throughout), and having Low/Normal (<18 ng/ml) or High (≥18 ng/ml) prolactin. Rates of normal cycling, acyclicity and irregular cycling were 73.2, 22.5 and 4.2% for Asian, and 48.4, 37.9 and 13.7% for African elephants, respectively, all of which differed between species ($P < 0.05$). For African elephants, univariate assessment found that social isolation decreased and higher enrichment diversity increased the chance a female would cycle normally. The strongest multivariable models included Age and Enrichment Diversity as important factors for cycling among African elephants. The Asian elephant data set was not robust enough to support multi-variable analyses of cyclicity status. Additionally, only 3% of Asian elephants were found to be hyperprolactinemic as compared to 28% of Africans, so predictive analyses of prolactin status were conducted on African elephants only. The strongest multi-variable model included Age, Enrichment Diversity, Alternate Feeding Methods and Social Group Contact as predictors of hyperprolactinemia. In summary, the incidence of ovarian cycle problems and hyperprolactinemia predominantly affects African elephants, and increases in social stability and feeding and enrichment diversity may have positive influences on hormone status.



Abstract #49

A practical method for timed ovulation in the Persian onager (*Equus hemionus onager*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

There are <1000 Persian onager remaining in the wild, and ~150 in accredited zoological institutions. Artificial insemination (AI) may be useful in managing this small population globally to minimize inbreeding. The first successful AI in onagers in 2009 required regular handling for ultrasound examinations. A method for estrous synchronization in this species would alleviate the need for daily handling and provide a more feasible approach to AI. This study tested single-dose administration of Progesterone and Estradiol (Day 0; BioRelease P4, 1500mg + Estradiol, 75mg, IM, BETPharm, Lexington, KY, USA), followed by cloprostenol (Day 11; 25µg, IM, BETPharm) in six female Persian onagers (age 3-11y) to determine whether ovulation would occur within a narrow window of time. Reproductive ultrasounds and urinary progesterone and estrone conjugates were used to determine the efficacy of this protocol. Based on hormone analysis, onagers were at various stages of the estrous cycle at the time of first injection. Three of six onagers (50%) ovulated on Day 19 and all onagers ovulated between Days 18 and 22, determined by the presence of a corpus luteum and followed by a luteal phase of normal progesterone concentration and duration (11-15 days). These findings are similar to studies in domestic equids, indicating an effective method for estrus synchronization in the onager. Combined with an ovulation inducing agent such as deslorelin to ensure a tighter frame of ovulation, this protocol may be used to develop a timed AI protocol at institutions that do not have specialized handling facilities to enable regular ultrasound.



Abstract #60

Ultrasound examinations and plasma progesterone concentrations of the alien species *Trachemys scripta* during reproductive season in Italy.

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Topic: Reproduction

Presentation type: Oral

Abstract:

The slider turtle (*Trachemys scripta elegans*) is now widely distributed in most wetlands in Europe as effect of recent massive imports for the pet-market and frequent release of these exotic turtles in natural habitats. Reproduction of this species has been observed under Mediterranean climatic conditions but endocrine fluctuations during the reproductive period are poorly understood. Turtles appear capable of annual breeding when in appropriate habitats, but facing suboptimal environmental conditions some adults females may not reproduce in a given year. Aim of this study was to gain better understanding of the slider turtle reproductive cycle collecting serum samples and performing ultrasound examinations on 38 females housed at the Pistoia Zoo, Pistoia (Italy) in a female-male mixed environment during a reproductive season. Serum Progesterone (P) concentrations were determined by radioimmunoassay (RIA) (Seren et al, 1974), P extraction was performed with petroleum ether, self-made polyclonal rabbit anti-progesterone (1:20000 final dilution) antibodies were used and analysis was performed in duplicate. Serum P baseline concentration was 0.54 ± 0.64 pg/ml. P results were compared with ultrasounds data. Echography identified follicular activity in 16 subjects (P: 0.35 ± 0.4 pg/ml), 12 non cyclic females (P: 0.8 ± 1.33 pg/ml) and 10 subjects with 2 ± 1 eggs (P: 0.32 ± 0.33 pg/ml), synchronous nesting behavior was documented for 3 animals (1 ± 2 eggs/subject). P concentration rose dramatically ($p < 0.01$) after deposition followed with a quick decrease before shell deposition started (predeposition 0.60 ± 0.31 pg/ml, postdeposition 1.40 ± 1.05 pg/ml). This preliminary study identified P basal concentrations during reproduction of the slider turtle and contemporaneously verified the ovarian activity ecographically. This protocol result as simple association of diagnostic tools capable of detecting clinical reproductive problems in pets animals and useful as a starting point to gain a better understanding of the endocrine cycle of this species.



Abstract #86

Monitoring of reproductive steroid hormone levels in relation to sexual swelling patterns in wild female bonobos (*Pan paniscus*)

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Topic: Reproduction

Presentation type: Oral

Abstract:

In the absence of endocrine data, the swelling of genitalia in female nonhuman primates is often used as a proxy for periods of high fecundity. However, there is variability in the timing of ovulation in relation to female sexual swelling patterns across the primate order, and bonobos often display a markedly long maximum swelling phase (MSP). We examined how endocrine parameters that are indicative of ovulation are associated with sexual swellings in wild female bonobos (*Pan paniscus*), and the temporal relation between the MSP and ovulation. Data were collected at the Luikotale field site, Democratic Republic of Congo, spanning 36 months from 2010 to 2013. Observational data on sexual swellings (n=14 females) were used to characterize swelling cycles, and urine samples were collected every 1–2 days to monitor their ovarian activity. We measured urinary estrone and pregnanediol using LC-MS/MS, and used pregnanediol levels to determine the timing of ovulation in 34 cycles from a subset of 9 females. Additionally, the occurrence of ovulatory and anovulatory cycles was confirmed using commercial luteinizing hormone tests (LH-S strips, Verify Diagnostics). The duration of the MSP was highly variable, lasting from 1–31 days. Timing of ovulation varied considerably in relation to the onset of the MSP, resulting in a low probability of ovulation occurring on any particular day of a female's MSP. Ovulation occurred during the MSP in only 52.9% of the analyzed cycles, revealing that the sexual swellings of female bonobos are less reliable indicators of ovulation compared to other species of primates. These findings highlight the importance of endocrine data to accurately assess the timing of ovulation in bonobos rather than relying on sexual swelling patterns alone. The temporal inflation and variability of these visual sexual signals in relation to ovulation may constrain mate guarding efforts by male bonobos.



Abstract #107

Fecal cortisol metabolite levels in black and gold howler monkeys (*Alouatta caraya*) inhabiting disturbed and non-disturbed areas of the humid Chaco region, Argentina

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Topic: Stress

Presentation type: Oral

Abstract:

In the last few years, the study of how environmental stimuli influence the physiology and specifically the endocrinology of an organism became increasingly important, relying mainly on the quantification of glucocorticoids to monitor animal welfare. Most studies of cortisol levels in primates were focused on the impact of social stressors. However, one of the major concerns for the conservation of howler monkeys is the increased habitat fragmentation led by the advancement of the agricultural frontier.

In this work we compared cortisol metabolite levels in howler monkeys (*Alouatta caraya*) living in continuous and fragmented forests of the Argentine humid Chaco region, throughout the warm season. Fecal samples (n=114) were collected from habituated and identified adult individuals, and extracts analyzed with an enzyme immunoassay also validated in this work (cortisol R4866, Department of Population Health and Reproduction, Coralie Munro, UC Davis, USA).

No significant differences were found in the levels of fecal cortisol metabolites in individuals inhabiting continuous vs. fragmented habitats (mean±SE in ng/g: 4160±244.3 (n=10 individuals) vs. 3573±242.9 (n=7 individuals); p=0.29); the slightly higher concentration detected in individuals of the continuous forest could probably reflect the high number of tourists visiting the Chaco National Park. The lower cortisol metabolite levels found in individuals inhabiting fragmented forests could be the result of low levels of disturbance imposed by a moderate and selective logging, which has proved to be beneficial for this species with high resilience by adjusting their diet to cope with feeding in degraded habitats but with new leaves and buds.

On the other hand, and regardless of the habitat, cortisol metabolites were significantly higher in females than in males (4318.1±211.5 ng/g (n=12 individuals) vs. 3031.9±250.6 ng/g (n=5 individuals) respectively; p=0.007).

Understanding how habitat quality impacts the physiological responses in primate populations is essential to build viable conservation models compatible with farming activities.



Abstract #80

Is adrenocortical activity associated with predation risk and anti-predator behaviour? A field test in free-ranging banded mongooses.

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Topic: Stress

Presentation type: Oral

Abstract:

Increased glucocorticoid production has been linked to predation risk and implicated in sub-lethal predation effects (depressing prey reproduction). Adrenocortical activity may thus mediate trade-offs between reproductive investment and anti-predator behaviours, by liberating energy reserves and facilitating increased vigilance, decreased foraging, predator evasion (flight response), use of predation refugia, and agonistic behaviour (e.g. mobbing). Hence, wildlife should generally exhibit positive associations between levels of glucocorticoids and anti-predator behaviours. To test this, we examined levels of faecal glucocorticoid metabolites (FGMs) in 13 groups of free-ranging banded mongooses (*Mungos mungo*) in northern Botswana (1542 samples, 268 animals, 2008 – 2010), measuring extracted immunoreactive FGMs using a physiologically-validated enzyme immunoassay detecting 11,17-dioxoandrostanes. At the population level, we determined how well proxies for predation risk (group size – predation risk dilution; using refugia from aerial predators) could explain variability in FGM levels, relative to other factors implicated in adrenocortical activity (reproduction; food limitation). Food limitation and not predation risk best explained variability in FGM levels. Food limitation had seasonal and anthropogenic components. Thus, we used separate seasonal analyses of groups with anthropogenic food sources, to test directly for associations between FGM levels and anti-predator behaviours, which we measured using Giving Up Densities (experimental foraging plot assays), and behavioural observations (vigilance, group spread [predation risk dilution], flight responses, mobbing). We found weak associations between levels of FGMs and anti-predator behaviours, usually in the dry season only. For example, mongooses had lower FGM levels when showing higher propensity to forage ($p \ll 0.05$, adj. $R^2 = 0.19$). We conclude that predation risk elicits negligible adrenocortical activity relative to food limitation in banded mongooses. At finer spatio-temporal scales FGM levels may be weakly associated with anti-predator behaviours, but only during dry seasons when food limitation may impose steeper behavioural, reproductive, and physiological trade-offs in preventing allostatic overload.



Abstract #116

Cortisol analysis in hairs of captive lynx species

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Topic: Stress

Presentation type: Poster

Abstract:

The use of hair cortisol as a marker of long-term stress is increasingly suggested as a non-invasive monitoring tool for wildlife animals and was recently applied in Canada lynx. In order to establish a hair sampling procedure in captive lynx, we determined hair cortisol levels in distal and proximal (2-3 mm fluff) samples collected from the inner surface of hind legs from 18 Iberian lynx (*Lynx pardinus*), 4 bobcats (*L.rufus*) and 2 Eurasian lynx (*L. lynx*) during veterinary examinations. In addition, the adrenal cortex was visualized by 3D-ultrasound allowing the determination of cortex thickness (C) in relation to the medulla (M).

Methanolic extracts (30 min) from hair samples obtained after washing and milling were subjected to an in house cortisol assay. Cortisol levels in distal hair (A) were strongly correlated ($p < 0.0075$) to levels in fluff samples (B). In the Iberian lynx, we determined for A 53.6 ± 4.5 pg/mg and for B 43.4 ± 2.9 pg/mg, in the bobcats A was 85.5 ± 4.6 pg/mg and B 74.3 ± 3.6 pg/mg. In the two Eurasian lynx A was 54.2 pg/mg and 61.0 pg/mg, whereas B was 46.7 pg/mg and 59.4 pg/mg, respectively. Analysis of correlation between hair cortisol and adrenal ultrasound in Iberian lynx reveal a correlation to C:M which was related to a behavioral score in a previous study. Interestingly, hair cortisol level of bobcats were significantly higher compared to other lynx, probably indicating insufficient housing conditions or a species-specific difference. Samples from two Iberian lynx collected after one year revealed 2-fold higher concentrations. The reason for this increase has to be elucidated. In conclusion, our preliminary study on hair cortisol in captive lynx indicated higher cortisol level in distal hair samples compared to samples from proximal fluff. Different cortisol levels might reflect changes in captive management; however more data are needed to verify this situation.



Abstract #50

Non-invasive assessment of adrenocortical function in Western Hoolock gibbon (*Hoolock hoolock*) by measurement of glucocorticoid metabolites in faeces

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Topic: Stress

Presentation type: Poster

Abstract:

There are numerous threats to the free-ranging Western Hoolock gibbon populations, including human habitat encroachment, hunting for food and traditional medicine, capture for trade, and forest degradation, resulting in an overall population decline of more than 90% with less than 5000 individuals remaining. Therefore, the species is currently listed by the IUCN as endangered, and captive breeding programs have been established to stabilise overall numbers. To evaluate stress-related physiological responses linked to housing, our aim was to examine the suitability of a group-specific enzyme-immunoassay (EIA) detecting 11,17-dioxoandrostanes for monitoring adrenocortical function in captive male and female Western Hoolock gibbon (*Hoolock hoolock*) based on faecal glucocorticoid metabolite (fGCM) analysis.

We collected a total of 176 faecal samples (27-30 samples per animal) over a month (Dec, 2014 – Jan, 2015) from 2 adult male and 4 adult female Western Hoolock gibbons housed at the Assam state zoo cum botanical garden, Guwahati, India. The animals were initially housed in either male-female pairs or same-sex (female) group, but one pair were separated due to frequent fighting and subsequently were housed individually throughout the study. The injured and thus separated female received medical treatment (© Melonex; Intas Pharmaceutical Ltd.) four-times during the monitoring period.

The female receiving painkillers had significantly lower overall fGCM levels compared to the other five individuals ($H_5=56.2$; $P<0.001$; post-hoc (Dunn's method) $P<0.05$). Overall median fGCM levels for the male and female gibbon kept as a pair were 64% and 68% higher, and median fGCM concentrations for the 3 singly housed individuals were 105%, 130%, and 144% higher, respectively. These findings provide support that the EIA system used in this study detects stress-related changes in circulating glucocorticoid levels in male and female Western Hoolock gibbons, and therefore represents a useful non-invasive tool for measuring physiological stress response in this species.



Abstract #87

Salivary cortisol secretion in semi-feral pigs under natural weaning conditions

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Topic: Stress

Presentation type: Poster

Abstract:

Intensive husbandry systems differ largely from natural living conditions with the weaning process representing one of the most stressful events in the pig's life. In addition to significant effects on health, growth and behavior, weaning has been associated with increased cortisol secretion. In natural environments, weaning occurs gradually and has been observed between 10 to 17 weeks of age. We aimed to investigate patterns of cortisol secretion and the onset of weaning in semi-feral pigs under natural living conditions.

Three farrows of semi-feral domestic pigs (of the Kune Kune breed; N=18) from two weeks of age until 20 weeks of age were included in the study. Saliva samples were collected weekly from each individual at two timepoints (9-10 am; 2-3 pm). To absorb pig saliva, commercial hydrocellulose/cotton swab devices were gently placed into the pig's mouth for 40-60 seconds. Prior to analysis, samples were stored at -20°C. Salivary cortisol was analyzed with a double-antibody biotin-linked enzyme immunoassay (Palme & Möstl, 1997). Moreover, the piglets' suckling behavior was monitored daily.

Preliminary analysis of our data reveals that characteristic circadian patterns of cortisol secretion were reached by the 10th week of age. The onset of weaning was observed at 12 (N=12) and 13 weeks of age (N=6) and accompanied by an increase in cortisol secretion. GLM analysis showed that cortisol levels during week 13 (4.4 ± 0.6 ng/ml, $Mn \pm SEM$) were significantly higher than during week 15 (2.6 ± 0.4 ng/ml; $p=0.33$) and 17 (2.5 ± 0.6 ng/ml; $p=0.45$).

Our preliminary findings are in line with earlier studies regarding the timeframe of the natural onset of weaning. We were able to demonstrate that also in free-ranging pigs, weaning seems to be associated with increased cortisol levels. The study is currently being continued with another cohort of piglets.



Abstract #51

Non-invasive stress measurement during road transportation of semi-feral Kune Kune pigs: A pilot study

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Topic: Stress

Presentation type: Poster

Abstract:

Short-term stress responses associated with transportation have been well-described in various livestock including pigs. It has also been suggested that an animal's response to handling and restraint during transportation is largely determined by previous experience. We aimed to measure stress responses non-invasively by analysis of salivary cortisol in semi-feral pigs. Moreover, salivary Neutrophil Gelatinase-Associated Lipocalin (NGAL) was monitored as a potential additional marker of stress-related immune reactivity.

As part of a major 3-years project on domestic pigs (of the Kune Kune breed) focusing on the socio-cognitive abilities when kept in a semi-natural environment and in a natural social system, three semi-feral gilts and one boar had to be transported for 90 minutes to a novel housing environment. Saliva samples from each individual were collected at resting conditions (baseline), before and after loading on the livestock trailer and after arrival at the destination (recovery). To absorb pig saliva, commercial cotton swab devices were gently placed into the pig's mouth for 40-60 seconds. Prior to analysis, samples were stored at -20°C. Salivary cortisol was analyzed using an antibody against cortisol-3-CMO:BSA and cortisol-3-CMO-DADDOO-biotin as label (Palme & Möstl, 1997) and NGAL was determined with ELISA (Pig NGAL ELISA Kit, BioPorto).

Salivary cortisol levels increased twofold in one individual, sixfold in two individuals and tenfold in one individual from baseline to post-transportation, reflecting the timepoint during road transportation. During the recovery phase, cortisol levels decreased, reaching baseline values. In contrast, NGAL baseline levels were higher compared to post-transport.

Our findings corroborate earlier results of increased cortisol levels during road transportation in pigs. Interpretation and generalization of the results is however limited by the small sample size and further research is needed to evaluate NGAL as a stress-sensitive immune marker.



Abstract #92

Glucocorticoid metabolites in wild capture-stressed White-tailed Ptarmigan in Glacier National Park, USA

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Topic: Stress

Presentation type: Poster

Abstract:

Due to high avian mobility and difficulties in collecting samples, understanding the glucocorticoid response following a stress event in wild birds can be problematic. The White-tailed Ptarmigan is an alpine grouse that has a combination of limited movement and prolific defecation and therefore is an excellent subject for studying glucocorticoid metabolites (GCM) in the wild. We obtained an initial fecal sample from wild White-tailed Ptarmigan in Glacier National Park, MT, USA, and then captured most, but not all of the individuals. Birds were released and followed for three hours while fecal samples were collected. A cortisone enzyme immunoassay (Rettenbacher *et al.* 2004) was used to measure fecal GCM. Baseline to peak increase (%) in GCM values was significantly higher in captured individuals ($x = 1932 \pm 696$; $N = 24$) than in individuals who had fecal samples collected but were not captured ($x = 138 \pm 61$; $N = 4$; $t_{23} = 2.1$; $P = 0.02$). Time to peak GCM averaged 66 ± 8 minutes for all captured individuals. However, several individuals reached peak GCM within 45 minutes including one with 1469% increase in 30 minutes. Ten individuals showed two peaks in GCM values, likely reflecting urinary and fecal components of the avian dropping. The second peak ($x = 149.7$ minutes) occurred over 1.5 hours after the first ($x = 53.3$ minutes) and wasn't significantly different in increase above baseline (percent increase of second peak: ($x = 360 \pm 102.7$; $t_{18} = 2.1$; $P = 0.21$). Due to the short time between stressor and GCM response, fecal samples make a viable alternative to plasma for understanding the response to acute stressors in this species. However, interpreting GCM values from wild birds can be complicated by the brevity of the peak response and the potential for both urinary and fecal peaks.



Abstract #93

Dosages of corticosterone in chinstrap penguins (*Pygoscelis antarctica*): effect of capture and physical restraint measured at three times.

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Topic: Stress

Presentation type: Poster

Abstract:

A stressful stimulus often leads to a rapid physiological response in individuals, contributing to their survival and the maintenance of homeostasis. Capture, physical restraint and manipulation are significantly stressful events, causing secretion of the stress hormone corticosterone (CORT). Studies indicate that the levels of CORT start to increase between 3 and 5 min after exposure of the organism to a stressful event. This study aims to investigate the impact of capture and physical restraint on the stress level of chinstrap penguins (*Pygoscelis antarctica*). We measured the concentration of CORT in blood samples collected (5 min, 30 min and 60 min after capture) in five adult penguins resident in Almirantado Bay, King George Island, South Shetland Islands, Antarctica. A corticosterone radioimmunoassay kit (MP Biomedicals, INC, CA-USA) was used. CORT levels increased with time in *P. Antarctica* (5 min = 0.93 ± 0.56 ng/mL; 30 min = 6.4 ± 3.14 ng/mL; 60 min = 9.16 ± 3.30 ng/mL). Corticosterone concentrations differed significantly between 5 min and 30 min ($p = 0.005$). However, no statistically significant difference between 30 min and 60 min was observed ($p = 0.2114$). These results indicate that the response to these stressful events is best seen 30 to 60 minutes after exposure. We are planning further studies considering other variables such as age, sex and sexual maturity to better understand the physiology of stress in *P. antarctica*.



Abstract #103

Analysis of corticosterone levels at different stages of reproductive biology in Antarctic Skuas (Catharacta spp.)

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Topic: Stress

Presentation type: Poster

Abstract:

Research on Antarctic seabirds, especially in Almirantado Bay, generally are limited to studies on distribution, abundance and general aspects of the species of a given area. The main goal of the present study was to investigate and understand the aspects of reproductive biology and stress levels of species Antarctic Skuas (*Catharacta lonnbergi* and *C. maccormicki*). We evaluated the basal and altered levels of serum corticosterone in *Catharacta* spp. and compared corticosterone values in the birds immediately after their capture and after 30minutes of capture and restraint. We sampled adults in reproductive and non-reproductive stages, chicks in different growth phases, and we compared results between sexes and species. There were significant differences ($p < 0.001$) in basal ($23.17 \pm 13.73 \text{ ng/mL}^{-1}$) and altered ($51.71 \pm 14.28 \text{ ng/mL}^{-1}$) serum corticosterone levels for adults, and higher levels following capture and restraint; but no significant differences between sexes ($p > 0.05$) and species ($p > 0.05$). Reproductively active adults had significantly higher basal levels than non-reproductive adults ($34.26 \pm 12.56 \text{ ng/mL}^{-1}$ and $14.55 \pm 6.63 \text{ ng/mL}^{-1}$; $p = 0.005$, respectively). These results indicated that serum corticosterone can be used as measure to identify altered levels of stress of such as capture and restraint of the individuals, but increase serum corticosterone in animals of reproductively active may be increased energy need during this phase. Next steps are to investigate and understand the aspects of reproductive biology, behavioral ecology and stress levels of Antarctic seabirds, emphasizing Antarctic Skuas.



Abstract #78

Progesterone fecal metabolites in the study of owl monkeys (*Aotus azarai infulatus*) estrous cycle

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Topic: Reproduction

Presentation type: Poster

Abstract:

Owl Monkeys are considered excellent experimental models and can contribute to the development of biotechnologies of reproduction in primates. Monitoring the reproductive cycle is a basic procedure, however complex in wild animals. Noninvasive methods have been developed to assess the reproductive hormonal profile of these animals expanding knowledge on reproductive physiology of primates in captivity and the wild. This study aims to monitor fecal progesterone levels to increase knowledge about reproductive physiology of this species. 12 adult females belonging to the breeding colony of owl monkeys of National Primate Center – CENP/Brazil were used. The study was performed using the 154 feces samples collection for monitoring the levels of metabolites of progesterone by enzymeimmunoassay (EIA). By progesterone assay we may only suggest the cycle of the species for the period studied ($16,7 \pm 4,6$ days). However, the baseline levels (2.1 ± 0.8 ng/g), mean peak (36.6 ± 8.6 ng/g), mean levels (4.7 ± 1.8 ng/g) and the minimum (0.4 ng/g) and maximum (49.9 ng/g) metabolites of progesterone were determined for the specie in captivity. The determination of the peak and basal levels of progesterone is noninvasive and feasible in the evaluation of the estrous cycle in owl monkeys.



Abstract #81

Fecal progesterone monitoring to demonstrate the efficacy of melengestrol acetate (MGA) implants as a reversible contraceptive for primates.

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Topic: Reproduction

Presentation type: Poster

Abstract:

Melengestrol acetate (MGA) implants are used as a contraceptive in zoo-housed primate species for population management. Contraception using MGA in a howler monkey (*Alouatta caraya*) and reversal following MGA implant removal in one Angolan colobus (*Colobus angolensis*) and three Guereza colobus (*Colobus guereza*) were documented using fecal progesterone monitoring. Prior to implant removal, the Angolan colobus and the Guereza colobus were implanted for 24 and 31-32.5 months, respectively. Progesterones were ethanol-extracted from fecal samples collected throughout the monitoring period and were analyzed by enzyme immunoassay using the R4859 antibody (University of California, Davis, CA, USA). MGA was effective in down-regulating progesterones in the howler monkey, and 14 days after implantation non-luteal progesterone concentrations decreased from $3.06 \pm 1.73 \mu\text{g/g}$ to $0.30 \pm 0.06 \mu\text{g/g}$ with no evidence of cyclicity over the last 7 months. Resumption of estrous cyclicity following implant removal was demonstrated 86 days later in the Angolan colobus by onset of luteal cycles and by pregnancy 55 days after implant removal for one Guereza colobus and ~6 months after implant removal for the remaining two animals. Samples were not available prior to implant removal, however non-luteal and luteal progesterone concentrations were $0.94 \pm 0.27 \mu\text{g/g}$ and $2.49 \pm 1.14 \mu\text{g/g}$ for the Angolan colobus following implant removal. Interestingly, progesterone concentrations for the Guereza colobus did not dramatically increase following implant removal and were measured at 0.46 ± 0.16 ? $3.67 \pm 0.82 \mu\text{g/g}$ before and 1.39 ± 0.67 ? $4.0 \pm 1.9 \mu\text{g/g}$ after implant removal. Nonetheless, gestation concentrations averaged $11.24 \pm 5.78 \mu\text{g/g}$. MGA was an effective contraceptive for these primate species, but time to resumption of cyclicity or pregnancy in the females was variable, and the possibility for reversal to require several months should be considered in management plans.



Abstract #44

Non-invasive monitoring of the effect of GnRH analog deslorelin acetate as a suppressant of the ovarian cyclic activity in captive common marmosets (*Callithrix jacchus*)

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Topic: Reproduction

Presentation type: Poster

Abstract:

With the purpose to evaluate deslorelin acetate, a GnRH analogue, as a reversible contraceptive, promoting the suppression the ovarian cyclic activity in captive *Callithrix jacchus*, the following method was used: Five couples of adult *C. jacchus* were divided in two groups: Deslorelin Group (DG), composed of three couples and Control Group (CG), with two couples. The experiment was executed in three sequential phases, as follows: Phase 1 (\pm 90 days/158 samples), confirming cyclic activity of all females. Phase 2 (\pm 120days/161 samples), DG females received one subcutaneous implant each, containing 2.35 mg of deslorelin acetate, while females from CG, received no implants (anticipating immediate surge of progesterone concentrations, followed by cyclic suppression). Phase 3 (\pm 90 days/126 samples), implants were surgically removed (awaiting return to cyclic activity). The non-invasive monitoring of the ovarian cyclic activity was done by first extracting fecal samples (dry feces) with 80% methanol, followed by measuring the concentrations of fecal monoclonal antibody anti-progesterone CL 425. Based on the concentrations of fecal progesterone metabolites (FPM), all females of both groups showed ovarian activity and ovulation during the entire studied period. DG females, after implant application, presented a pronounced elevation of the FPM (mean value/dev 440,030 ng/gr during phase II), which was expected due to an initial stimulation of the Hypothalamus-Pituitary-Gonadal axis. Nevertheless, the anticipated decrease in FPM caused by down-regulation of GnRH receptors apparently did not occur, hence, no cessation of ovarian activity, to the contrary, a stimulatory effect could be observed. Leading to postulate that concentrations of 2.35 mg deslorelin was insufficient to promote the desired effect. Furthermore, CG females demonstrated similar FPM values (mean value/dev 411,269 ng/gr during phase II), opening speculations of possible tendency toward synchronization of ovarian cycles, as females where housed in close proximity and common air flow, permitting of olfactory cues.



Abstract #101

Detection of Progesterone in Fecal Samples of a Nonbreeding Female Jaguar via High Performance Liquid Chromatography

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Topic: Reproduction

Presentation type: Poster

Abstract:

The jaguar (*Panthera onca*) is one of the most recognizable felids in the world but little has been published on the species' reproductive physiology. In order to characterize their estrous cycle it is important to evaluate steroid metabolite profiles in fecal samples. The objective of this study was to investigate the presence of progesterone metabolites in fecal samples from female jaguars throughout the estrous cycle. Fecal samples from a reproductively intact, nonbreeding female jaguar were collected for two consecutive months and analyzed via high performance liquid chromatography (HPLC). Before the analysis, the samples were lyophilized, pulverized, and a hormone extraction was performed by adding 80% methanol (10 mL) to 2.0 g of powder in 15-mL plastic tubes, and homogenizing the samples overnight. After centrifugation, 5 mL of the supernatant were purified in a C-18 cartridge. Hexane was added to the purified solution to separate the food dye used for sample identification. The dye-free fraction was evaporated, resuspended in 400 mL 80% methanol, and analyzed via HPLC. Efficient purification was obtained using a binary system chromatographer (CBM-20A SHIMADZU, Prominence-LC-6AD) equipped with a detector (UV-Vis, model SPD-20A), with data acquisition performed via a microcomputer, and an analytical column (LiChroCART® 250-4 LiChrospher® 100 RP-18 endcapped, 5 µm, Merck) attached to a C-18 pre-column. Chromatographic conditions were as follows: 20-mL injection volume, isocratic solvent system of acetonitrile:water (50:50 v/v), 1.0 mL/min flow rate, wavelength of 235, and a 30-minute analysis. Chromatograms revealed a peak that had the same retention time (~27 min) as the progesterone standard indicating that progesterone in its original chemical structure is present in fecal samples from a nonbreeding female jaguar. Given that jaguars are believed to be induced ovulators further analyses are needed to confirm if this progesterone is of follicular origin. Financial support: FAPESP (#2013/12757-9) and CAPES.



Abstract #65

A preliminary study of non-invasive monitoring of reproductive hormones in fecal samples from captive Sunda Pangolins (*Manis javanica*)

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Topic: Reproduction

Presentation type: Poster

Abstract:

Sunda Pangolins (*Manis javanica*) are listed as 'critically endangered' by the IUCN. The population is in steep decline due to intense poaching for their meat and scales. Very little is understood about the reproductive physiology of pangolins. They are elusive in the wild and only a few studies have been conducted on captive individuals. Hormonal investigations of *ex situ* populations are therefore essential for successful implementations of captive breeding programs. The objective of this study was to determine the possibility of using non-invasive fecal metabolites to understand the reproductive hormone patterns of the Sunda Pangolin at Wildlife Reserves Singapore.

For this study, fecal samples were opportunistically collected from four non-pregnant, adult female Sunda Pangolins that were housed individually. Samples were dried and extracted with 90% ethanol. Progesterone and Estrone Conjugate (EC) metabolite concentrations in the extracts were analyzed by laboratory validated enzyme immunoassays (EIA) using antibodies against Progesterone (CL425, UC Davis) and EC (R522-2, UC Davis) respectively. Serial dilutions of fecal extracts were parallel to standard curves for both assays examined.

Preliminary results indicated a mean EC concentration of 40.1 ± 16.2 ng/g feces and progesterone concentration of 3.9 ± 2.1 ug/g feces among three female pangolins, which are suspected to be non-cycling. The current breeding female on the other hand, showed erratic spikes in EC concentration of up to 330.1 ng/g feces from a mean concentration of 76.4 ng/g feces. No clear indication of a luteal phase had been determined by the progesterone EIA.

Results suggest that EC and progesterone concentrations can be obtained by non-invasive sample collection, indicating the feasibility of using fecal metabolites to examine reproductive hormone levels in Sunda Pangolins. However, the study recognizes limitations in interpreting these results, given the brief sampling period. A more extensive study is currently underway to determine a comprehensive hormonal profile for this species.



Abstract #99

Reproductive and adrenal function in injured or diseased male koalas (*Phascolarctos cinereus*)

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

In south-east Queensland, hundreds of koalas are admitted to wildlife hospitals each year, primarily due to domestic dog attacks, motor vehicle trauma or disease. Chlamydia is widespread in south-east Queensland koalas and is associated with significant health and reproductive issues. This project has been developed to increase our understanding and monitoring of the relationship between disease and/or trauma injury and reproductive and adrenal function in male koalas. Fecal samples were collected from koalas, admitted and treated at three wildlife hospitals, extracted in 80% MeOH and analysed for corticosterone (FCM) and testosterone (FTM) metabolites by enzyme-immunoassays (antibodies sourced from C. J. Munro, UC Davis, USA). Preliminary data includes fecal hormone metabolite concentrations over two to twelve weeks from 10 adult male koalas (n = 8 disease; n = 2 injury). FTM concentrations and profiles varied between individuals (overall mean 352.1 ± 161.4 ng/g); low animal numbers prohibiting trend detection. FCM concentrations were also highly variable (overall mean 388.4 ± 250.7 ng/g) but the three koalas with the lowest mean concentrations (114.9 to 129.7 ng/g) were also the least variable ($P < 0.05$), possibly an indicator of chronic adrenal depression or altered adrenal function. These koalas could be considered the most extreme hospital cases of the current study animals. They included significant trauma injury or chlamydia related conjunctivitis and cystitis in addition to minor injuries and were in care for one to three months prior to sampling. Expansion of the data to include additional koalas of varying levels of injury and disease, sampled over extensive periods of rehabilitation and treatment in comparison to healthy, captive koalas, will increase our understanding the aetiology of chlamydia infection in koalas as well as facilitate the development of an index of reproductive and adrenal function for future *in situ* and *ex situ* research studies.



Abstract #79

Reliably measuring urinary neopterin level variation as a health status indicator in bonobos and chimpanzees

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

Variation in health status has an important impact on survival, reproduction and ultimately overall fitness and is therefore important in studies of behavioural ecology. One way to assess an individual's health status is by detecting the presence of an immune response. During a cell-mediated immune response, interferon- γ is released by the activated T-cells stimulating the monocytes / macrophages to produce neopterin, thereby leading to a measurable increase in the urinary concentration of this biomarker in humans.

To test the viability of a commercially available human neopterin ELISA assay (Neopterin ELISA, IBL, Ref. RE59321) for use on chimpanzees and bonobos urine, we measured 62 samples (1 - 6 per individual) from 18 bonobos (Nmale = 8; Nfemale = 10) and 10 samples from chimpanzees (Nmale = 5; Nfemale = 5) from 11 zoos.

In bonobo and chimpanzee urine pools, neopterin levels in serially diluted samples paralleled the standard curve. Average neopterin recoveries after the addition of exogenous neopterin were between 75% and 122%, and urine pool samples stored at room temperature for 48 h showed a decrease of urinary neopterin levels of around 20% in both species. There were no diurnal changes in urinary neopterin profiles of eight bonobos where samples were collected at 7 am, 12 pm, and 5 pm. Using a within - subjects comparison, eleven bonobos showed significantly higher urinary neopterin levels with a bacterial infection than when healthy. On average, urinary neopterin increased by a factor of five in sick individuals. However, urinary neopterin levels overlapped across sick and healthy individuals.

Neopterin can be reliably measured in the urine of bonobos and chimpanzees and may serve as a biomarker for the detection of infections in bonobos. However, baseline samples from healthy individuals are needed for comparison to assess health status.



Abstract #47

Behavioral and hormonal responses associated with forage availability in captive western lowland gorillas (*Gorilla gorilla gorilla*)

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Topic: Animal health and welfare

Presentation type: Oral

Abstract:

The availability of forage material was systematically manipulated for three male western lowland gorillas (*Gorilla gorilla gorilla*) at the Detroit Zoo. Over four consecutive two-week conditions, forage was provided for four (baseline), two, seven, and four (baseline return) days a week. We predicted that greater forage availability would increase feeding-related behaviors and activity, and decrease concentrations of c-reactive protein (CRP), a biomarker of inflammation associated with sedentary behavior and its metabolic/cardiovascular risks in humans. We validated a commercial ELISA (Salimetrics, USA) for gorilla salivary CRP: a serial dilution ($n = 4$) of pooled saliva showed parallel displacement to the standard curve (ANCOVA, $F_{1,12} = 1.283$, $p = 0.280$), and recovery values for control-spiked sample pools were 105% (high) and 116% (low). Intra- and inter-assay coefficients of variation were less than 10% and 15%. There was a significant main effect of condition on CRP (repeated measures ANOVA, Wilks' lambda = 0.564, $F_{3, 37} = 9.551$, $p < 0.0001$) and behavior (Wilks' lambda = 0.618, $F_{51, 667.69} = 2.293$, $p < 0.001$). The gorillas spent more time processing food when forage was available daily, and only during this condition was regurgitation and reingestion absent. The gorillas spent more time sitting when forage was offered only twice weekly, but overall mobility was unaffected by condition. Significant changes in CRP were due to one gorilla's health problem during the study, suggesting the assay has biological validity but the browse intervention may not have impacted CRP. Finally, an ELISA (Arbor Assays, USA) was validated for fecal glucocorticoid metabolites (FGM): serial dilutions ($n = 6$) of pooled samples showed parallel displacement to the standard curve ($F_{1,10} = 2.505$, $p = 0.145$), and recoveries were 113% (high) and 94% (low). FGM results will be discussed with other findings and their implications for zoo gorilla welfare.



Abstract #114

Serum testosterone, free testosterone, cortisol and lipid profile during different stages of musth in captive Asian elephants (*Elephas maximus*)

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Topic: Animal health and welfare

Presentation type: Poster

Abstract:

Musth is a unique physiological phenomenon in male Asian elephants (*Elephas maximus*), characterized mainly by heightened aggressive behavior. In southern India, more than a thousand captive elephants are maintained in different management systems. To facilitate proper musth management, a preliminary evaluation of serum testosterone, free testosterone, cortisol and complete lipid profile was done in 11 captive elephants. Single blood samples were collected from nine male elephants in different stages of musth and two female elephants. In the nine males, based on apparent physical and behavioral changes like engorgement of temporal glands and perineum and aggression toward mahouts, one was in non-musth, one in pre-musth, two in musth and five in post-musth. Serum testosterone (electrochemiluminescence assay using monoclonal antibodies; Cobas®), varied between 0.01 and >15 ng/ml; the lowest values were in the two females and the highest value was in a male in musth. Free testosterone (ELISA using monoclonal antibodies; Diametra®), varied between 0.0001 and 0.014 ng/ml; the lowest values were in the two females and the highest values were in the two males in musth. Serum cortisol, (chemiluminescence assay using monoclonal antibodies ; Cobas®), varied between 4 and 30 ng/ml; the lowest value was in a female and the highest values were in two males, one in pre-musth and the other in musth. Cholesterol (mean 48; 28-60 mg/dl), serum triglycerides (mean 13.3; 9-22 mg/dl), LDL-cholesterol (mean 31.6; 14-42 mg/dl), HDL-cholesterol (mean 14; 7-19 mg/dl) and VLDL-cholesterol (mean 2.7; 1-4 mg/dl) were measured by enzymatic colorimetric method (Cobas®). The one time sample collection could not differentiate the variations in lipid profile between either the males in different stages of musth or the males and females or the male/female age classes. From this preliminary evaluation, close monitoring of free testosterone along with serum cortisol seem to help in early detection of musth.



Abstract #53

Serum testosterone and cortisol as they relate to behavior among group-housed bull African elephants (*Loxodonta africana*).

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Topic: Animal health and welfare

Presentation type: Poster

Abstract:

Housing African elephants in bachelor groups is an efficient use of space and mimics naturally occurring social groups in the wild. To assess relationships among four African bull elephants housed together (age = 31, 11, 11, 6 years), serum cortisol and testosterone concentrations were evaluated weekly for two years by enzyme immunoassay using the R4866 and R156/7 antibodies (University of California, Davis, CA, USA), respectively. Behavioral data, including the initiator, recipient, and outcome, were recorded using continuous focal animal sampling during one year. Bulls were classified as either active or passive if their hourly initiated interaction frequency was greater or less than the mean frequency for all of the bulls. Dominance was determined by calculating the percentage of the total antagonistic interactions that resulted in a “win” for each bull in every possible dyad. The two active bulls that frequently sought both affiliative and sparring interactions had lower ($p < 0.05$) cortisol (25.3 ± 0.8 and 27.2 ± 1.2 ng/ml) than the two passive bulls (33.0 ± 1.0 and 37.3 ± 1.3 ng/ml). The oldest bull was the dominant bull, however neither testosterone nor cortisol was closely associated with dominance. The dominant bull experienced musth once when testosterone increased from 5.2 ± 0.3 to 46.0 ± 7.1 ng/ml, indicating that the absence of female stimuli and the continuous presence of other bulls did not prevent this natural event, but testosterone was highest in the dominant bull only during musth. Appropriate adrenal functionality in these bulls was demonstrated by variability in the cortisol profile without evidence of chronically-sustained elevations. These endocrine and behavioral evaluations demonstrate that bachelor group management of African elephants can be appropriate, and a bull's social activity might have implications for measured cortisol concentrations.



Abstract #111

Case report: state of health of two spectacled bears (*Tremarctos ornatus*) in the natural reserve "La Planada", Narino - Colombia

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Topic: Animal health and welfare

Presentation type: Poster

Abstract:

In April 2014 veterinarians from the University of Nariño (Colombia) carried out a clinical evaluation of two spectacled bears (*Tremarctos ornatus*) of the Natural Reserve "La Planada" Nariño-Colombia, kept in captivity for education, conservation and eco-tourism Purposes. The evaluation was conducted to assess the health status of the animals and to perform medical management and corrective treatments if necessary. The bears were anesthetized with ketamine (7 mg/kg) and xylazine (1 mg/kg) administered with a dart gun. We performed a general clinical examination, evaluation of hematological and blood chemistry parameters and serology of some zoonotic pathogens in each animal. Radiographs and abdominal and pelvic ultrasounds were also taken. Clinical examination revealed apparent good health in the two bears. Although through the white blood cells results (neutrophilia and lymphopenia) bad condition of the two animals was evident. Moreover, high levels of cortisol were observed: 14 mg/dL and 15,7 mg/dL (Reference: 5,8 mg/dL), which are associated with stress processes that induce immunosuppression and infectious diseases prevalent in bears that are kept under conditions of poor complexity of captivity. Calcium (7.75 and 5.72 mg/dL) and phosphorus (3,7 mg/dL and 2,6 mg/dL) also showed low values (Reference: 8,6-10 mg/dL and 3.6-7.1 mg/dL, respectively) that were in agreement with the periosteum, osteolysis and loss of vertebral bone density found radiographically. Overall, although this is a report with only two animals, our findings suggest nutritional deficiencies due to improper feeding and highlight the need to foster welfare and health in captive animals of this threatened species.



Abstract #90

Prediction of faecal cortisol metabolites from western lowland gorilla (*Gorilla gorilla gorilla*) by near infrared reflectance spectroscopy (NIRS)

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Topic: Methodologies

Presentation type: Oral

Abstract:

Quantification of faecal cortisol metabolites as a reflex of the hypothalamic-pituitary-adrenal axis activity has been widely used to assess stress in animals. EIA and RIA are the most employed techniques for hormone quantification. Although they have a good sensitivity and specificity, they require a hormone-extraction procedure, the samples cannot be further used and the assay procedures are time-consuming. Conversely, near infrared reflectance spectroscopy (NIRS) is a rapid, reagent-free and non-destructive technique, but needs to be calibrated by standard laboratorial methods. The aim of this study was to evaluate the feasibility of using the NIRS technique to predict the quantification of faecal cortisol metabolites from western lowland gorillas (*Gorilla gorilla gorilla*). Eighty-four faecal samples from 2 males and 2 females were collected as a part of a global project of gorilla wellbeing in zoos and stored at -20°C. For the analysis, samples were dried in an oven at 60°C for 48h. A portion was used for cortisol metabolites determination by an anti-cortisol EIA kit (Neogen, Ayr, UK) after a methanol-based extraction and other portion was used for NIRS determination. Seventy-three faecal samples were used for NIRS calibration and the eleven remaining samples for external validation. NIRs values were correlated with EIA ones by modified partial least squares regression (MPLS). The coefficient of determination (R²) was 0.92, the coefficient of determination for validation (r²) was 0.80 and the ratio of performance deviation (RPD) was 2.5. The RPD is a non-dimensional statistic for the evaluation of a NIRS calibration model and is defined as the ratio of standard deviation of the reference values for the standard error of prediction. Determined RPD values between 2.5 and 2.9 providing a fair prediction can be used for sample screening. This preliminary study suggests that NIRS is a promising tool for hormonal detection in faeces from gorillas, with lower cost and time consumption than conventional methods.



Abstract #98

Inhibitory action of thymol in fecal microbial activity of *Tamandua tetradactyla* and its effect on glucocorticoid metabolite measurements

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Topic: Methodologies

Presentation type: Poster

Abstract:

Measuring fecal glucocorticoids have potential to improve wildlife conservation, but there are methodological concerns. Feces require storage methods to avoid steroid degradation by fecal microorganisms. Freezing is recommended; however, it is a costly method under non-controlled environmental conditions. This study aimed at determining if thymol reduces the proliferation of microorganisms in feces from *Tamandua tetradactyla* and affects hormone measurements. Fresh feces were collected from each animal (n=5), divided into fractions (5.5g each) and kept in sealed glass Petri dishes at 22±2°C. Treatment consisted of a thymol solution (5mg g⁻¹ feces; 500 µL; 80% ethanol), whereas control consisted of 500 µL of the ethanol solution. Additionally, negative control for hormone measurements consisted of samples without ethanol solution. All samples were evaluated at 0, 24, 48 and 72 h post-excretion. Total aerobic and anaerobic heterotrophic mesophilic bacteria, spore-forming bacteria (SFB), total coliforms, mould and yeast were counted. For hormone analysis, samples (0.5g) were frozen and then dried (60°C). The analysis of variance indicated that thymol had an inhibitory effect on four of the five studied groups of microorganisms, with a significant reduction of up to three orders of magnitude being observed in the colony forming units per gram of feces at 24, 48 and 72 h. By contrast, SFB show no reduction induced by thymol. Unexpectedly, when analyzing the effect of post-excretion time on hormone measurements, treatments show no differences across time periods. More studies should evaluate if feces could be maintained without freezing up to 72h post-excretion. Thymol affected hormone measurements, however, values were more similar to negative control than control (negative control: 1251±118 > thymol: 986±98 > control: 792±77 ng/g dry feces; p=0.05). Thymol might be useful to control microbial activity in feces from other species; however, other solvents should be evaluated to avoid inaccuracy in hormone measurements.



Abstract #77

hormLong: An R package for the longitudinal analysis of hormone data

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Topic: Methodologies

Presentation type: Oral

Abstract:

Longitudinal hormone monitoring yields valuable information about endocrine physiology. However, analysis of longitudinal hormone data often requires extensive use of graphical and numerical analyses. Though graphical analysis of longitudinal profiles are essential for understanding longitudinal data, the creation of tens, if not hundreds, of graphs can be time-consuming. Furthermore, routine numerical methods for summarising profiles, such as the iterative baseline approach and area under the curve (AUC), can be tedious and non-reproducible, especially for large number of individuals. To facilitate the production and analysis of longitudinal hormone profiles, we have developed an R package (hormLong) that provides the basic functions to perform graphical and numerical analyses routinely used by wildlife endocrinologists. R is a free program, thereby making this program readily accessible to all users. To encourage its use, hormLong has been developed such that little to no familiarity with R is necessary. Here, we will provide an overview of the package and the functions currently available. We hope that this package will promote reproducibility and facilitate analysis of longitudinal hormone data.



Abstract #56

Oxytocin modulates proximity and checking behaviours in phocid seals

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Topic: Misc

Presentation type: Poster

Abstract:

Endogenous plasma oxytocin concentrations correlate with proximity behaviour exhibited by wild grey seal (*Halichoerus grypus*) and human (*Homo sapiens*) mothers. Intravenous (IV) oxytocin manipulations were given to newly weaned grey seals in a semi-natural trial arena to determine if elevated oxytocin concentrations caused changes in the distance individual seals maintained from each other. Pairs of individuals ($n=21$) that had never been in contact before were given either an IV oxytocin or saline injection. Behaviours such as the frequencies of olfactory and visual investigations ('checks'), and the time spent in close proximity (less than one body length) to each other were recorded. After the trial, individuals were separated and, after a rest day, the same pair was reunited in the trial arena. Each individual was manipulated with whichever injection they did not receive during the first trial, to control for significant individual behavioural variation that exists in this species. Plasma samples were taken before and after a subset of trials, and oxytocin concentrations were measured using an ELISA (Enzo Life Sciences, Lausen, Switzerland) previously validated for detecting plasma oxytocin in phocid seals.

Individuals given oxytocin injections prior to trials spent greater amounts of time in close proximity to each other ($p=0.004$) and performed less 'checking' behaviours ($p<0.001$) than those given saline manipulations. Plasma samples taken post-oxytocin manipulation showed significant elevation of plasma oxytocin concentrations ($p<0.001$) when compared to basal and post-saline plasma samples, despite the rapid clearance rate of peripheral oxytocin and an hour elapsing since the injection. This indicates we developed and used a suitable dose to penetrate the blood brain barrier and affect central brain regions. This study is the first to demonstrate that manipulating oxytocin directly impacts on behaviours that have been correlated to endogenous oxytocin concentrations in the wild.



Abstract #75

Melatonin excretion in photoperiodic mammalian and bird species: chinchilla and Japanese quail.

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Presentation type: Poster

Abstract:

Photoperiod is a powerful synchronizer of seasonal changes in physiology and behavior. Melatonin (Mel) is considered a zeitgeber for seasonal photoperiodic changes and plays a role in energy expenditure and body mass regulation. Our studies explore the possible influence of Mel in stress and reproductive responses to environmental cues, in photoperiodic species such as chinchilla (*Chinchilla lanigera*) and Japanese quail (*Coturnix coturnix japonica*). To develop a reliable non-invasive method to monitor pineal function, we designed an experiment to generate basic information about melatonin excretion, determining its main route and time course of excretion, determining its main route and time course of excretion. Labeled Mel (¹²⁵I-Mel; 5 μ Ci/male) and unlabeled Mel (25 μ g / 100g b.w.) were intraperitoneally injected to separately housed males (n=3 in each group). Excretes were collected from each individual before (-24h) and after ¹²⁵I-Mel injection (every 2 h during the first day and every 12 h until 96 h were reached). Samples were stored at 20 °C until processing. After ¹²⁵I-Mel administration in chinchilla, a radioactive peak appeared in the first urine samples (range: 2-6 h post-excretion) and rapidly decreased; in feces, maximum radioactivity exhibited a median of 8 h post-administration (range: 6-34 h). Most of the radioactive metabolites were recovered in urine (median 92.65 \pm 2.64%). In quail, peak excretion occurred at 3 h (range 2- 4 h). In all birds, a second smaller peak was observed between 8 and 12 h; total recovery was 90.57 \pm 5.57%. In conclusion, the excretion pattern was similar in males of both photoperiodic species. Chinchilla employed mainly urine as a route to excrete melatonin. Quail exhibited two radioactive peaks in excreta, presumably representing urine and feces excretion. Future research is necessary to characterize excreted metabolites of endogenous melatonin in order to develop and/or choose the appropriate immunoassay.