INTERNATIONAL SOCIETY of WILDLIFE ENDOCRINOLOGY

PROCEEDINGS

of the 8th ISWE CONFERENCE

6-9 November 2023

Corbett National Park, India

Hosted by Ahmedabad University, Center for Cellular & Molecular Biology, and Wildlife Institute of India

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Wildlife Institute of India

Welcome to the 8th Conference of the International Society of Wildlife Endocrinology!

A very warm welcome to the 8th Conference of the International Society of Wildlife Endocrinology, hosted by Ahmedabad University, Center for Cellular & Molecular Biology, and Wildlife Institute of India. It seems like a long four years since we last met in-person, and I know the challenges of the pandemic and other global events continue for many. For those of us attending, it will be a great opportunity to see some familiar faces, meet new, and network with our international colleagues. Following the success of our virtual event in 2021, for the first time we are combining an in-person conference held at the Riverview Retreat, Corbett National Park, India, and an online virtual event, using the Whova platform; so, for those of you who can't attend in person, we are glad you can still join us with the wonders of technology!

This year, 65 colleagues from 13 countries registered for our in-person conference, plus an additional 43 colleagues registered on our virtual platform, totaling 108 participants from 18 countries. We have an exciting program with 38 oral and 18 poster presentations to share with you, along with three fantastic plenary speakers. Dr. Maria Thaker from the Centre for Ecological Sciences, Indian Institute of Science will be providing an interesting insight into stress, sex and danger: the behavioural endocrinology of lizards in a challenging world; Dr. Janine Brown from the Smithsonian National Zoo and Conservation Biology Institute will share a brief history of wildlife endocrinology and ISWE: a personal perspective of lessons learned; and Dr. Samir Sinha, a member of the Uttarakhand Cadre of the Indian Forest Service will be joining us to discuss conservation efforts and management challenges of Corbett Tiger Reserve. We look forward to hearing from all our presenters on topics including biomarkers of physiologic stress, integrative animal health, reproductive function, long-term studies of wildlife and new techniques for applied endocrinology. As well as the scheduled presentations, we will be holding a best-practices workshop to discuss some of the common practices of wildlife endocrinology and take advantage of the ISWE hive-mind to help us develop some guiding principles and advance our field even further. A new addition to our program this year is the Mentor-Mentee Trivia Night – this has been organized by our Trainee Affairs Committee, and should be a fun way to meet others, all with a little light competition thrown into the mix! We'll also have our usual ice-breaker, conference dinner and poster sessions, so please take these opportunities to network with colleagues and share ideas.

Earlier this year we held a contest to design our ISWE8 logo; we had some beautiful entries and thank everyone that entered for sharing their creativity with us. Dr. Liz Burgess created the winning entry, so thank you, Liz, for the incredible tiger design that captures ISWE and our venue so perfectly! We would also like to thank our 29 ISWE colleagues for reviewing abstracts and travel awards. This year we were able to offer more awards than ever before, thanks to sponsorship from our partners at Arbor Assays and a grant from the Morris Animal Foundation. Congratulations to our Arbor Assays, Coralie Munro, ISWE emerging nations and Morris Animal Foundation travel grant awardees, our six recipients of conference registration waivers, and our two virtual event presentation winners from 2021, who are back to share more of their research with the community. We look forward to hearing from you all!

A huge thank you has to go to our conference hosts, Dr. Ratna Ghosal, Dr. Govindhaswamy Umapathy and Dr. Samrat Mondol, for welcoming us to India and arranging this beautiful venue, and to our conference chairs Dr. Diana Koester and Dr. Jella Wauters, who together with our hosts, have worked tirelessly behind the scenes getting us all here and ready for what I'm sure will be an inspiring few days.

Finally, we thank all of you for attending the conference, whether in-person or via our virtual platform. ISWE prides itself in being a collaborative community and with every year is becoming increasingly international in its reach – we hope to inspire our members, both old and new, to advance the field of wildlife endocrinology by stimulating discussion and generating new ideas to help us study and protect wildlife, and you are all an important part of our journey.

We hope you all enjoy our conference!
Katie Edwards, ISWE Chair

ACKNOWLEDGMENTS

We express our sincere thanks to the following colleagues for reviewing abstracts or judging travel awards (indicates individuals that did both*):**

Cayman Adams
Camryn Allen
Meredith Bashaw
Megan Brown
Elizabeth Burgess
Tina Chiarelli*
Elizabeth Donelan
Katie Edwards*
Kerry Fanson*
Elizabeth Freeman
Grace Fuller
André Ganswindt
Ratna Ghosal
Rebecca Hobbs
Kathleen Hunt
Tamara Keeley
Diana Koester
Shana Lavin*
Lara Metrione*
Samrat Mondol
Anneke Moresco
Linda Penfold
Natalia Prado-Oviedo
Beth Roberts*
Rachel Santymire
Juan Scheun
Karen Steinman
Govindhaswamy Umapathy
Jella Wauters

Our Conference Planning Subcommittee
Jella Wauters – Conference Chair
Diana Koester – Outgoing Conference Chair
Katie Edwards – proceedings, moderators, travel awards
Tina Chiarelli – registration assistance, finances
Tamara Keeley – conference communications support
Beth Roberts – conference communications and website support
Samrat Mondol – host, shuttle arrangements, visa applications
Govindhaswamy Umapathy – host, materials selection/coordination
Ratna Ghosal – host, venue logistics, sponsor relationships/applications
Tshepiso Majelantle – mentor/mentee night, Whova, shirts
Allie Case – mentor/mentee night
Lara Metrione – sponsor relationships
Linda Penfold – sponsor relationships
Emmy Hirsch – Save the Date graphic design
Elizabeth Burgess – Conference logo design
Madeline Winans – Whova app support
Valentina Melica – Whova app support

Session Moderators
Elizabeth Burgess
Allie Case
Tina Chiarelli
Brinky Desai
Katie Edwards
Ratna Ghosal
Kathleen Hunt
Tshepiso Majelantle
Valetina Melica
Samrat Mondol
John O'Hanlon
Beth Roberts
Govindhaswamy Umapathy
Andrea Webster

We warmly thank our invited speakers for contributing keynote sessions:

PLENARY SPEAKERS
Dr. Janine Brown – Smithsonian National Zoo and Conservation Biology Institute. A BRIEF HISTORY OF WILDLIFE ENDOCRINOLOGY AND ISWE: A PERSONAL PERSPECTIVE OF LESSONS LEARNED.

Dr. Samir Sinha – Indian Forest Service, Uttarakhand Cadre. CONSERVATION EFFORTS AND MANAGEMENT CHALLENGES OF CORBETT TIGER RESERVE.

Dr. Maria Thaker – Indian Institute of Science. STRESS, SEX AND DANGER: THE BEHAVIOURAL ENDOCRINOLOGY OF LIZARDS IN A CHALLENGING WORLD.

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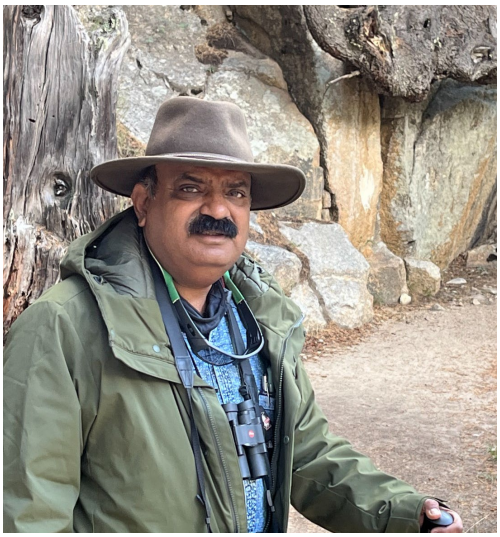
PLENARY SPEAKER: DR. MARIA THAKER

Maria Thaker is an Associate Professor at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore India. Her lab takes an integrative and comparative approach to understand phenotypic form and function, especially in lizards. This involves exploring multi-trait variation within and between species, the functional connection between morphology, behaviour, physiology, and nutrition, as well as the evolutionary patterns of these trait complexes. Her lab is particularly interested in understanding the implications of environmental change, such as urbanization, and climate change, on such animal responses. Most recently, her lab is focused on how fear, foraging, and sex is modulated by stress.

PLENARY SPEAKER: DR. SAMIR SINHA



Samir Sinha is a member of the Uttarakhand Cadre of the Indian Forest Service (IFS). Currently he is the Principal Chief Conservator of Forests (Wildlife). And Chief Wildlife Warden for the state of Uttarakhand. He has over 30 years of experience in Natural Resources and Wildlife Management, and he has served as the former Directors of Corbett Tiger Reserve, Rajaji Tiger Reserve and Nanda Devi Biosphere Reserve. He has also served as the Former Head of TRAFFIC India. He was a Fulbright Nehru Fellow and received his PhD in Wildlife Science. He has helped establish and operationalize the South Asian Wildlife Enforcement Network (SAWEN) for strengthening regional cooperation for wildlife enforcement. He brings a unique combination of science-based conservation and management leading to effective wildlife policy and law. He has forged strong collaborations with various government and non-government organizations to deal with human-wildlife conflict in Uttarakhand.

PLENARY SPEAKER: DR. JANINE BROWN

Dr. Janine Brown is a wildlife endocrinologist devoted to better understanding the biology of wildlife species and factors that affect reproduction, welfare, and health. She is currently Head of the Endocrine Research Laboratory at the Smithsonian Conservation Biology Institute (SCBI) and has trained and/or mentored over 100 fellows, students, and interns both in the U.S. and abroad in basic and applied research methods, specifically in the areas of endocrinology and theriogenology. Research efforts are connected to the scientific disciplines of behavior, endocrinology, and stress management, with a special emphasis on elephants, where she plays a key role in ex situ management of both species in the U.S., and Asian elephants in range countries. With over 35 years of experience in wildlife endocrinology, Dr. Janine Brown saw firsthand how hormone monitoring grew to become an integral part of conservation physiology science. She was an early adopter of noninvasive techniques to measure hormones in urine, feces, hair, and saliva, resulting in the largest wildlife endocrine database, and training of dozens of students/visiting scientists. To further promote the field, she co-founded ISWE in 2011 to bring specialists together from around the globe to share endocrine experiences. Through examples from three plus decades of work, she will present a brief history of wildlife endocrinology and the genesis of ISWE.

Arbor Assays Student Travel Scholarship:
Alicia Dimovski
Ph.D. Student, La Trobe University

Alicia Dimovski's research investigates the impact of artificial light at night on Australian nocturnal mammals. Her research characterizes the effect of light spectra on nocturnal mammal activity and physiology and evaluates options for "wildlife-sensitive" lighting. Alicia has monitored circadian hormones (melatonin and glucocorticoids) in a range of Australian mammals using non-invasive urine and fecal samples. Her research shows that artificial light disrupts melatonin and glucocorticoid expression, however the effects are not consistent across species. Alicia's research demonstrates that species' behavior and physiology is altered by light and highlights both the need, and solutions for, "wildlife sensitive" lighting.

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Morris Animal Foundation Emerging Nation Travel Scholarship:
Shiv Patel
Wildlife Sciences Ph.D., Wildlife Institute of India

Shiv Patel's broad area of research interest is understanding the impact of changing environments on wild animals and populations, how they adapt, survive, or thrive, largely through a physiological lens, and using that to inform conservation decisions. For more than 5 years, she has been working on the stress physiology of large felids in their natural environment in the Terai-Arc landscape, India. She helped her supervisor establish a wildlife endocrinology laboratory at the Wildlife Institute of India. For her Ph.D., she delved into understanding the impact of ecological and anthropogenic factors on the physiological health status (fecal glucocorticoid and triiodothyronine measures) of wild tigers inhabiting a gradient of habitat in terms of quality at a landscape scale. She has also investigated physiological aspects of species interaction, that is, the impact of dominant tigers on socially subordinate leopards at a spatio-temporal scale. She does interdisciplinary research utilizing ecophysiological, GIS, telemetry, and genetic tools.

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Morris Animal Foundation Emerging Nation Travel Scholarship:
Andrea Webster
Ph.D., Mammal Research Institute, University of Pretoria

Dr. Webster's research combines years of practical experience in the field with expertise in ecology, animal behavior and behavioral endocrinology which she has applied to non-invasive research techniques and analysis. More recently Dr Webster is applying these skills to the development of non-invasive ways to assess endocrine disruptors from environmental pollutants in wildlife, particularly mammals to assess ecosystem health. In addition to this research, she is currently investigating the diet of scavenging carnivores in the arid Kalahari, South Africa using stable isotope analysis. Andrea has also contributed to popular articles for Africa Geographic, the Conversation and Oppenheimer Research and Conservation. She has been hosted as a visiting researcher with the Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany, the Animal Physiology and Behavior team, Faculty of Tropical Agrisciences, Czech University, Prague and recently received attended advanced training in multiple aspects related to endocrine disruption at the Marine Biological Laboratory, Woods Hole, MA, USA.

Coralie Munro Memorial Travel Scholarship:
Emily Potratz
Graduate Student Researcher, The University of Illinois at Chicago

Emily Potratz's research aims to address wildlife management questions, from abundant species such as white-tailed deer, to rare and endangered species such as black-footed ferrets. She uses physiological and behavioral indicators of stress and reproduction to understand why some populations thrive and why some struggle. The connecting thread among her projects is individual and population level hormone load. She measures glucocorticoids, androgens, and estrogens from hair and feces, respectively, that she then relates to behavior and other physiological metrics such as body condition, gonadal changes, and gut health. These indicators complement one another to paint a more complete picture of the animal's internal and external state. Emily hopes to continue wildlife conservation work that incorporates an evolutionary perspective to better understand how all animals, including humans, can thrive in the changing world.

ISWE Emerging Nation Student Travel Scholarship:
Brinky Desai
Ph.D. Student, Ahmedabad University

Brinky Desai's doctoral research focuses on understanding patterns, processes, and endocrine correlates of reproductive behavior in freshwater mugger crocodiles. She has also developed a biometric system to identify individual mugger crocodiles using UAV imagery. For the last four years, Brinky has been spending a large part of her time in remote areas within India to collect scat samples from mugger crocodiles, and to conduct long hours of behavioral observations. She is also an expert in flying and deftly maneuvering the UAV over muggers to collect their images for focal identification. When not in the field, she spends time in the lab either programming the biometric codes or conducting hormone assays to analyze the mugger scats for a better understanding of their reproductive patterns. Aside from her research work, Brinky also runs an Early Career Crocodile Network (ECCN) along with Dr. Phoebe Griffith from Oxford University, UK, and is also a member of IUCN's Crocodile Specialist Group.

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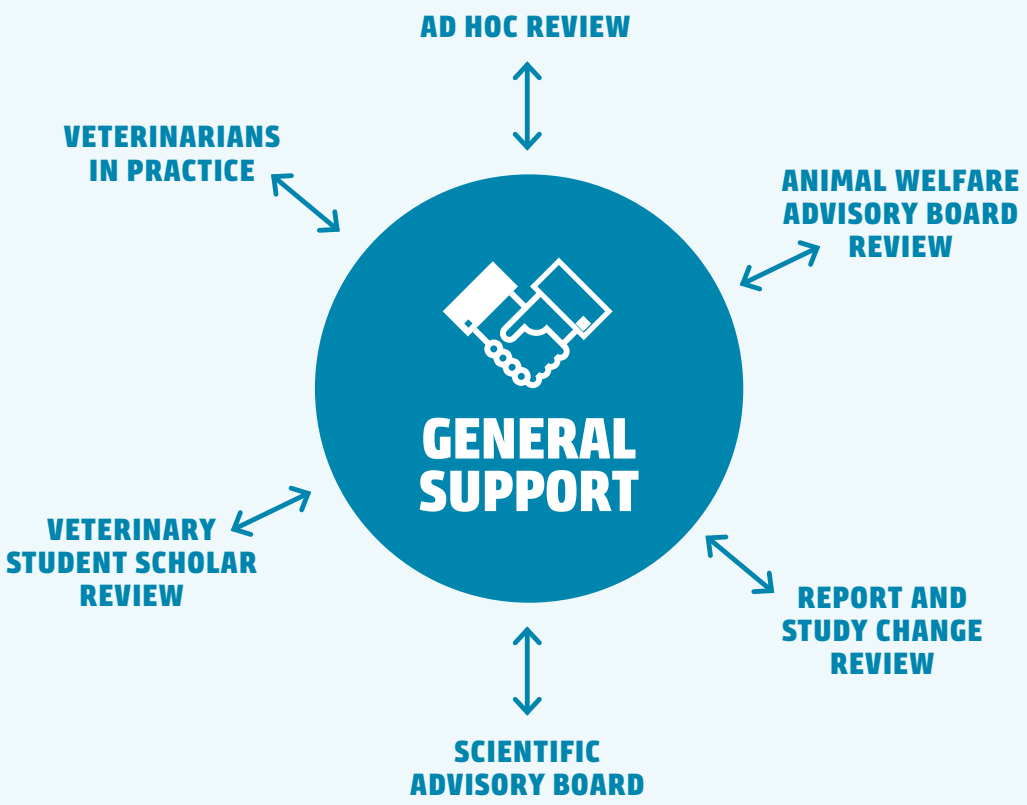

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Page	Presenting author	Abstract title
	Brinky Desai	Non-invasive monitoring of stress-indicating hormone in mugger crocodile (*Crocodylus palustris*) across diverse habitats: validation of fecal glucocorticoid assay for application in a free-ranging population
	Jacqueline Morrison	Fencing for conservation: physiological and demographic responses to barriers to dispersal in an African elephant (*Loxodonta africana*) population
	Kelly Williams-Kelly	Physiological response after translocation differs between source population types in a threatened mammal
	Grace Fuller	Feather corticosterone as an indicator of feather condition and experiences during molt of zoo-housed crested penguins (*Eudyptes spp.*)
	Kathleen Hunt	Hormonal variation and temporal dynamics of musth in zoo-housed Asian elephants (*Elephas maximus*) are associated with age, body condition, and the social environment
	Andrea Webster	Integrative animal welfare: combining behavioural endocrinology with ecotoxicology
	Emily Potratz	Using reproductive hormonal, physiological, and behavioral indicators to inform on breeding in the black-footed ferret (*Mustela nigripes*)
	Rebecca Evey	Reconstructing glucocorticoid and reproductive history of critically endangered Rice's whales using baleen
	Beate Braun	AKR1D1 and CYP21A2 – Hidden players in steroid metabolism of corpora lutea of felids?
	Loraine Shuttleworth	A new insight into southern right whale reproduction via baleen endocrine and stable isotope analysis
	Shiv Patel	New paradigm in tiger conservation: landscape-scale physiological correlates of various natural and anthropogenic factors across Terai-Arc landscape, India
	Jen Jelincic	Longitudinal adrenal, reproductive, and thyroid hormone profiles from four 1960's bowhead whales
	Allison Case	Profiling the hidden whales of the Smithsonian: longitudinal hormone analyses of Antarctic baleen whales from the WWII era
	Katherine Fowler	The lion's share of the lions' hair: insights into free-ranging lion stress and reproductive physiology using hair hormone analysis
	Samrat Mondol	The cost of sympatry: spatio-temporal patterns in leopard dietary and physiological responses to tiger competition gradient in Rajaji Tiger Reserve, Uttarakhand, India
	Tshepiso Majelantle	Non-invasive quantification of reproductive hormone metabolites after a gonodotrophin-releasing hormone administration in the naked mole-rat (*Heterocephalus glaber*)
	Amy Miller	Characterization of basic reproductive biology in Amur leopards (*Panthera pardus orientalis*) using non-invasive monitoring
	Rebecca Mogey	How suppressed is suppressed? Long-term application of progestin as a contraceptive in giraffe
	Michal Mateusz Hryciuk	Development of a 3D cell culture system to investigate mechanisms regulating corpus luteum functionality in felids

Valentina Melica	Lipid content and hormones: how to get the most out of a single extraction
Maeve O'Keeffe	Selecting your solvent: could it change your results?
Elizabeth Donelan	An efficient way to evaluate multiple biomarkers in serum samples
Emily Bovee	Validating a fecal DNA damage assay via comparison to glucocorticoid metabolites in captive wildlife
Alicia Dimovski	Does wildlife friendly lighting have a physiological cost? Investigating the impacts of LED lighting on melatonin and glucocorticoid expression in nocturnal mammals
Saverio Lubrano	The role of mesotocin in regulating social behavior in Siberian jays: insights from a wild population
Andre Ganswindt	Alterations in faecal glucocorticoid metabolite concentrations post-defaecation across three animal feeding classes (ruminants, hindgut fermenters and carnivores)
Kodzue Kinoshita	An attempt to evaluate the physiology of wild snow leopards using fecal steroid hormone analysis
Kerry Fanson	Expression of glucocorticoids across the ovarian cycle in the critically endangered mountain pygmy-possum
Heriberto Barbosa-Moyano	Adreno-immunological response in rehabilitation processes of ex-situ in tropical screech owl (*Megascops choliba*)
Sanjeeta Sharma Pokharel	Physiologically plastic elephants: highly dynamic glucocorticoid responses in African and Asian elephants
Tom Cools	Development and validation of a steroidomics methodology for non-invasive biomonitoring in wildlife
Kirsten Wilson	Preliminary use of urinary NIRS for early pregnancy detection in giant pandas
Elizabeth Burgess	Record-breaking mortality of Florida manatees: can fecal hormone assessment help provide early warnings of population consequences?
Camryn Allen	All eggs in one poor quality basket: assessing Hawaiian green sea turtle (honu) resilience in light of climate change impacts
Hannah Roberts	Faecal androgen metabolites exhibit predictable fluctuations across the ovarian cycle in Tasmanian devils (*Sarcophilus harrisii*).
Lindsey Vansandt	Characterization of 13,14-dihydro-15-keto-prostaglandin f2-alpha metabolite (PGFM) in the sand cat (*Felis margarita*) and Pallas' cat (*Otocolobus manul*) as a non-invasive marker for pregnancy resulting from natural breeding or artificial insemination
Vinod Kumar	Non-invasive reproductive monitoring helps in successful breeding of mouse deer (*Moschiola indica*) in captivity
Tamara Keeley	Validation of non-invasive hormone analysis techniques to assist in the identification of maternal roosts of ghost bats (*Macroderma gigas*)
John O'Hanlon	Utilising routine non-invasive faecal samples for the detection of oestrus and early gestation in okapi (*Okapi johnstoni*)
Beth Roberts	Successful pairing, monitoring, and pregnancy detection of large spotted genet (*Genetta tigrina*)

Candace Scarlata	Longitudinal analysis of reproductive and adrenal hormones in Bontebok (*Damaliscus pygargus*): a case study of social changes and reproductive events
Nadia Gray	Assessing reproductive hormones in adult female blue whales (*Balaenoptera musculus*) by analyzing historic baleen samples from the 1940's
Piper Thacher	Calving intervals inferred from progesterone patterns in historic baleen of female fin whales (*Balaenoptera physalus*)
Ghulam Nabi	Changes in testicular histophysiology and immuno-intensity of androgen receptor in brown bear (*Ursus arctos*) in association with age, season, and spermatogenic score
Tathagata Bhowmik	Understanding context and endocrine correlates of aggressive behavior in female mugger crocodiles during reproductive and non-reproductive phases
Daniela Mello	Glucocorticoids as potential biomarkers of stress in sei whale (*Balaenoptera borealis*) baleen
Gudimella Anusha	Human activity and wildlife tourism: significant contributors to elevated glucocorticoid levels in *Panthera tigris tigris* (Tiger) in India
Tatsuki Shimamoto	Stress in the Anthropocene: the complex relationship between physiological stress and anthropogenic food consumption in sika deer
Katie Edwards	Development of an ISWE 11-oxoaetiocholanolone mini-kit for the non-invasive quantification of glucocorticoid metabolites in feces
Kaling Danggen	Quantifying corticosterone in bird feathers to assess chronic stress levels in avian populations inhabiting eastern Himalayan forests
Kimberly Todd	Behavioral and endocrine correlates in a reintroduced population of swift foxes (*Vulpes velox*)
Sukesh Bhupathy	Habitat-dependent foraging behaviour of Nilgiri langurs (*Semnopithecus johni*) and their stress status in the Nilgiris: a comparative preliminary study
Jane Braswell	A change of heart: seasonal trends in heart rate and hair thyroid hormones in maned wolves (*Chrysocyon brachyurus*)
Danielle Dillon	Studying hormones in one of the most elusive, deepest-diving species on the planet, Blainville's beaked whales (*Mesoplodon densirostris*)

Non-invasive monitoring of stress-indicating hormone in mugger crocodile (*Crocodylus palustris*) across diverse habitats: validation of fecal glucocorticoid assay for application in a free-ranging population

Brinky Desai[1], Tathagata Bhowmik[1], Rohith Srinivasan[1], Nikhil Whitaker[2], Ratna Ghosal[1]

[1] Ahmedabad University, Biological and Life Sciences, School of Arts and Sciences, Ahmedabad, India
[2] Madras Crocodile Bank Trust, Zoo, Chennai, India

Presentation type: Oral Presentation
Presenting author: Brinky Desai
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Abstract:
Monitoring physiological parameters like stress hormones can aid in understanding adaptation strategies of a species across diverse environments. The mugger crocodile is one such species that occurs in 50 small pockets in different habitats across India and the subcontinent. The objective of this study is to implement a validated, non-invasive method to measure fecal glucocorticoid metabolite (fGCM) levels for free-ranging populations of muggers inhabiting diverse habitats. We selected three habitats (defined as "a", "b", and "c") in central Gujarat, India: (a) habitat with medium human population and low conflict, and non-industrial zone, (b) habitat with high human population and medium conflict, and an industrial zone (c) habitat with low human population and high conflict, and an industrial zone. The fGCM measured used an assay targeting 5b-3α-ol-11-one structure that has previously been validated for Nile crocodiles (*Crocodylus niloticus*). We also carried out biological validation of the assay by collecting opportunistic fecal samples during annual medical examinations on restrained captive muggers at Madras Crocodile Bank Trust, Chennai, India. Our results showed an 11 times increase ($P<0.05$) in fGCM levels (Mean±SEM) from pre-capture 540.9±149.2 (n=11) to capture 6259.7±1150.5 (n=11) ng/g dry fecal samples, thus, validating the assay. The validated fGCM assay was applied to free-ranging populations (n =107 scats from habitats 'a', 'b' and 'c'). So far, preliminary analyses indicate fGCM levels were significantly different across habitats ($P<0.05$, One-way ANOVA), and with significant differences between habitat 'a' (368.2±66.6, Mean±SEM, n=16), the low conflict region, and the habitat 'c' (1431.8±377.4,Mean±SEM, n=7), the high conflict zone. The findings of the study can be utilized to understand adaptability of mugger populations across diverse habitats within their natural distribution across India.

Fencing for conservation: physiological and demographic responses to barriers to dispersal in an African elephant (*Loxodonta africana*) population

Jacqueline Morrison[1,2], Sue Walker[2], Rebecca Mogey[2], Katie L. Edwards[2], Bradley Cain[1]

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[2] North of England Zoological Society, Chester Zoo, Science Department, Chester, UK

Presentation type: Oral Presentation
Presenting author: Jacqueline Morrison
Corresponding author email: j.morrison@chesterzoo.org

Abstract:
Fencing of protected areas has become a necessary strategy to safeguard wildlife, prevent over-exploitation, and mitigate human-wildlife conflict. These physical barriers to movement lead to isolated, fragmented populations. Whilst the genetic implications of isolation are widely understood, there is a paucity of information on the physiological effects of curtailing dispersal in wide-ranging species, and what are the potential ramifications of these for population viability. Our objectives were to measure and compare concentrations of faecal glucocorticoid metabolites (fGM) in an African elephant (*Loxodonta africana*) population fenced since 2009, a free-ranging population, and another fenced population utilising a wildlife corridor to move across the wider landscape. Secondly, as long-term elevation of glucocorticoids has been associated with negative effects on reproduction, we recorded the demographic structure (age and sex ratios) of all of the study populations compared, and additionally to populations of known growth status. Overall concentrations of fGM in the fenced population (21.8 ± 9.1 ng/g) were significantly higher ($H = 32.5$, $df = 2$, $P < 0.001$) than the two control populations (14.8 ± 5.4 ng/g; 17.4 ± 7.6 ng/g), with no significant difference between the control populations ($Z = 1.82$, $P = 0.069$). The largest differences were seen in the adult age-class, with concentrations in the fenced population (21.96 ± 10.15 ng/g) significantly higher than all others ($H = 8.11$, $df = 2$, $P = 0.017$). Results from the demographic assessment identified a highly skewed age distribution in the fenced population, indicating a reduction in reproductive output. Age class frequencies were significantly different to populations of known stable ($\chi^2 = 215.98$, $P < 0.001$), and rapidly increasing growth status ($\chi^2 = 329.1$, $P < 0.001$). Understanding the physiological responses to restricted movement and potential influences on population viability can inform conservation management decisions and provide evidence for landscape connectivity.

Physiological response after translocation differs between source population types in a threatened mammal

Kelly S Williams-Kelly[1], Laurence Berry[2], Kim Branch[3], Saul Cowen[3], Sean Garretson[3], Greg J Holland[4], Rachel Ladd[4], Liberty Olds[5,6], Kelly Rayner[3], Colleen Sims[3], Leanne Van Der Weyde[3], Kylie A Robert[1], Kerry V Fanson[1]

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Presentation type: Oral Presentation
Presenting author: Kelly Williams-Kelly
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Abstract:
Conservation translocations are an important tool in preventing species loss by moving animals to facilitate healthy wild populations. However, translocations are inherently stressful events for animals. Stress can negatively affect reproduction and survival, influencing translocation success. Monitoring physiology provides valuable insights about how animals respond to translocation and can reveal strategies to mitigate negative impacts. Glucocorticoid metabolites in faeces present a non-invasive approach to monitoring stress physiology in wildlife. In this study, we compared physiological responses across three translocations of greater stick-nest rats (GSNRs), *Leporillus conditor*. We first validated an assay to measure faecal glucocorticoid metabolites (FGMs) in the species. We then compared pre- and post-translocation (1-5 months post-release) FGMs of three different source populations of GSNRs. The source populations comprised the last naturally-occurring wild population (remnant-wild), a wild population reintroduced ~35 years ago (reintroduced -wild) and a captive-bred population. Pre-translocation, the remnant-wild population had the lowest average FGM concentrations (46.6 ± 23.1 ng/g), followed by the captive-bred population (108.1 ± 24.3 ng/g), and the reintroduced-wild population had the highest (374.8 ± 14.9 ng/g). Post-translocation, FGMs of the remnant-wild population did not change, while the reintroduced-wild population exhibited a significant decrease ($p = 0.043$) and the captive-bred population a significant increase ($p<0.0001$). Our work highlights that different source populations can have vastly different physiological responses to translocation. Wild-sourced GSNRs acclimated better post-translocation, however, sourcing from wild populations is not always feasible. This emphasises the need for further study into factors affecting adaptation following translocation. Furthermore, methods are needed to maximise success for all source population types, such as selecting the most suitable individuals and high-quality release sites. We can improve translocation outcomes by better understanding how species and individuals respond to translocation, thereby positively contributing towards species conservation.

Feather corticosterone as an indicator of feather condition and experiences during molt of zoo-housed crested penguins (*Eudyptes* spp.)

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Presentation type: Oral Presentation
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Abstract:
Penguins undergo a catastrophic molt replacing all their feathers over an approximately two-week period once per year. We aimed to explore the utility of naturally molted feathers as an integrated measure of adrenal activity during this critical period for penguin welfare. We collected feathers from N=30 macaroni (*Eudyptes chrysolophus*) and N=25 southern rockhopper (*Eudyptes chrysocome*) penguins at the Detroit Zoo annually for five years as the birds moved between two exhibits. During this time, many individuals developed annual feather breakage occurring as early as 200 days prior to molt, with the severity and size of the affected areas increasing until molt. We monitored breakage using staff ratings and by measuring heat emission from affected areas using infrared thermography. Given that glucocorticoids have a degradative effect on proteins, we hypothesized that penguins experiencing more severe feather breakage would have higher concentrations of corticosterone in feathers (fCort). We performed a methanol-based extraction and analyzed fCort in 203 samples using an enzyme-immunoassay (CJM006 antibody, C. Munro; enzyme conjugate, J. Brown, Smithsonian) validated by parallelism: $y=0.86x+14.05$, $R^2=0.99$, $F_{1,5}=1735.99$ $p<0.0001$ for macaroni penguins and $y=0.63x+48.03$, $R^2=0.84$, $F_{1,6}=31.67$, $p=0.001$ for rockhopper penguins. Generalized linear mixed models indicated that individuals showing breakage over a longer pre-molt duration had higher log(fCort) concentrations in both macaroni ($F_{1,57}=6.57$, $p=0.01$) and rockhopper ($F_{1,24}=5.32$, $p=0.03$) penguins. For both species, older penguins tended to have higher fCort, but fCort was not related to exhibit or staff ratings. Thermal readings for macaroni penguins also did not predict fCort values. Macaroni penguins showed potential relationships between fCort and individual social behaviors, including agonism and social proximity. The causal factor underlying feather breakage in the colony remains unclear. However, these results suggest potential for fCort as a non-invasive measure of feather quality that also may be indicative of behavioral experiences during molt.

Hormonal variation and temporal dynamics of musth in zoo-housed Asian elephants (*Elephas maximus*) are associated with age, body condition, and the social environment

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Abstract:
Asian elephant populations are threatened with extinction, and male elephants pose unique challenges to long-term sustainability. “Musth,” a heightened sexual state in male elephants, is accompanied by a range of physiological changes, requiring special consideration when developing short- and long-term management strategies for elephants. The objective of this study was to characterize hormonal variation in relation to intrinsic (age, body condition, musth status) and extrinsic factors (social environment) in zoo-housed Asian elephants. We measured fecal androgen metabolites (FAMs) and fecal glucocorticoid metabolites (FGMs) via enzyme immunoassay from fecal samples collected weekly over approximately one year from 26 male Asian elephants housed in zoos across the USA. For elephants that exhibited musth, both FAM and FGM concentrations were higher during musth (average FAM = 394.17 ng/g dried feces, average FGM = 18.20 ng/g) compared to non-musth (FAM = 58.35 ng/g, FGM = 15.97 ng/g) periods. Further, FAM concentrations were associated with variation between age and body condition (age was inversely related to FAM concentrations at lower body conditions, but directly related at higher body conditions). Additionally, increased exposure to other males was associated with lower FAM concentrations, while increased social access to females was correlated with higher FAM concentrations. FGM concentrations were also associated with variation between age and body condition (FGM increased with age at high body conditions, while the opposite was true at low body conditions). Finally, the duration of musth was inversely related with body condition ($P = 0.045$) and directly related to exposure to other males ($P = 0.033$). These results indicate that hormonal profiles around musth vary significantly between male elephants, and this variation can be explained in part by intrinsic and extrinsic factors. By identifying factors that influence animal wellbeing and reproduction, studies like this help to improve the sustainability of Asian elephant populations.

Integrative animal welfare: combining behavioural endocrinology with ecotoxicology

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Presentation type: Oral Presentation
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Abstract:
Given the numerous components involved, reliably evaluating overall health of the environment and the wildlife it supports is complex. As a result, very few studies evaluate wildlife and environmental health simultaneously. Site-specific differences in geology, environmental conditions and anthropogenic activities as well as different legislation governing best practice at local, regional and global scales further complicate matters. The development of non-invasive methods to assess animal welfare in free-ranging and captive settings has provided numerous insights into the factors affecting animal welfare and reproductive health. A recent study conducted in the South African Kalahari, assessed faecal glucocorticoid metabolite (fGCM) concentrations in free-ranging porcupines for the first time. Using a set minimum increase of 100% as a target during biological validation, the 11 oxo-aetiocholanolone-I enzyme immunoassay detecting 11,17-dioxoandrostanes performed best. In comparison to captive individuals, free-ranging individuals demonstrated overall lower fGCM concentrations indicating that animals in the wild appear to be in overall good health. Soil biodiversity is considered as a primary factor in regulating the functioning of terrestrial ecosystems but are often a sink for anthropogenic contaminants such as trace elements. Toxin effects are typically dose-related, but endocrine disrupting chemicals, including potentially toxic elements (PTE) can disrupt normal functioning of the endocrine system at any dose. At the same site, we evaluated 10 potentially PTE in sediment, vegetation and a wide range of terrestrial wildlife including porcupines. Trace element concentrations were highest overall in sediment and when evaluated at species-level, multiple elements appear to be highest in fossorial species. Given these species come into regular contact with soil during foraging, burrowing and reproductive activities, they may be particularly sensitive to endocrine disruption. These studies highlight an opportunity for research innovation and collaboration between the fields of behavioural endocrinology and ecotoxicology.

Using reproductive hormonal, physiological, and behavioral indicators to inform on breeding in the black-footed ferret (*Mustela nigripes*)

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Abstract:
Abnormal behaviors and decreased fitness are unfortunate consequences of some *ex situ* breeding programs. For the endangered black-footed ferret (*Mustela nigripes*; hereafter ferret), the current captive breeding protocols may be leading to reproductive decline. The ferret has been managed for over 35 years, with pairs chosen using pedigree analysis to maintain genetic diversity. This singular focus may ignore the potential benefits from reinstating natural behaviors such as mate choice or even polyandry. To explore indirect aspects of mate choice we tracked the reproductive state of 48 breeding ferrets using hormonal and physiological traits and examined how both traits related to behavior and preference. We examined exploration, activity budget, and odor preference of potential mates across two breeding seasons using an olfactory-dominant behavior assay. Ferrets received odor cues from two different potential mates simultaneously in a modified T-maze. As female fecal estradiol (FEM; Munro R0008) and male androgen (FAM; Munro R156/7) metabolites increased, so did female exploration ($R^2 = 0.8$; $p = 0.032$) and male scent investigation ($R^2 = 0.45$; $p = 0.028$), respectively. Females closer to estrus also sniffed male odors more than pre-estrus females ($t = 2.95$; $p = 0.001$). For females, vaginal cornification was a more reliable predictor of estrous state than FEMs; likely due to large day-to-day hormonal variability. Neither sex showed preferences for individuals in higher reproductive states. Results indicate heightened interest by reproductive males and females in exploring and assessing spaces that may offer access to potential mates. Within breeding facilities, individuals may be receiving dozens of odor cues from conspecifics without the opportunity to act on them. Providing odor cues may be able to encourage mate choice behaviors, provide behavioral enrichment, and increase breeding success.

Reconstructing glucocorticoid and reproductive history of critically endangered Rice's whales using baleen

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Abstract:
The Rice's whale (*Balaenoptera ricei*) is a newly characterized species of baleen whale found in the Gulf of Mexico. With fewer than 50 adults remaining, the Rice's whale is the world's most endangered baleen whale species. Analysis of reproductive and adrenal hormones could assist in understanding stress physiology and reproductive cycles in this poorly understood species. Baleen plates contain steroid hormones stored throughout the period of baleen growth and have been used for continuous, multi-year retrospective assessment of reproductive and glucocorticoid history of individual whales. We measured progesterone, testosterone, cortisol, and corticosterone in baleen plates of seven individual Rice's whales (four males — one of which was the holotype — and three females), including two individuals believed to have died from starvation. Baleen powder was obtained by drilling every 1 cm (~15-30d intervals) from the base of the plates to the distal end. Hormones were extracted from the powder with methanol and concentrations quantified with commercial enzyme immunoassay (EIA) kits. All assays demonstrated parallelism for Rice's whale baleen extract. In the two individuals that died of starvation, all four steroid hormones show increases in the most recently grown baleen, a pattern observed in other baleen whales in cases of prolonged illness or injury before death. A female with a confirmed recent pregnancy had a pronounced elevation in progesterone throughout most of her baleen plate, indicating that baleen analysis in this species can detect recent pregnancies. No evidence of annual testosterone cycles was noted in three adult males, suggesting that this subtropical species might not have strongly seasonal reproduction. Baleen hormone analysis may help clarify endocrine history and causes of mortality in this critically endangered species.

AKR1D1 and CYP21A2 – Hidden players in steroid metabolism of corpora lutea of felids?

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Abstract:
Corpora lutea (CLs) are usually transient endocrine glands, producing progesterone. In contrast to other species, CLs of lynxes do not undergo complete regression; they persist. The present study is a sub-study following transcriptome analysis by RNA-sequencing of CLs from domestic cat of development/maintenance stage (dm, n = 8) and regression stage (re, n = 12) for characterisation CLs life cycle. Analysing differentially expressed genes, two steroid metabolizing enzymes were attracting our attention – aldo-keto reductase family 1 member D1 (AKR1D1) and steroid 21-hydroxylase (CYP21A2). Here, complementary to gene expression analysis via quantitative RT-PCR (qPCR), we checked the protein expression with western blot. To determine whether the expression pattern of these enzymes differs between lynxes and domestic cat, we studied the following lynx samples via qPCR too: 12 CLs of Iberian lynxes (two animals, from each 3 fresh – formations stage – and 3 persistent CLs – dm, d 7 after mating), 11 CLs of one Eurasian lynx (5 fresh CLs – dm, 6 persistent CLs – dm/early regression (er), d17 of pregnancy) and 6 CLs of three Eurasian lynxes (outside breeding season, er). In addition, we examined mass spectrometry data of lynx CLs for the protein presence of these enzymes. The qPCR as well as the protein analysis confirmed a much stronger expression of both enzymes in re-CLs compared to dm-CLs of domestic cat. Surprisingly, the expression was in persistent CLs of lynxes higher than in the corresponding fresh CLs, even if persistent CLs showed morphologically dm-stage. We assume that these enzymes could contribute to a local turnover of progesterone – CYP21A2 by catalyzing the 21-hydroxylation of progesterone and AKR1D1 by forming 5β-dihydrosteroids. Moreover, AKR1D1 could contribute to the metabolism of surplus cholesterol. Which specific metabolites are generated and whether they are suitable as markers, should be analyzed in future.

A new insight into southern right whale reproduction via baleen endocrine and stable isotope analysis

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Abstract:
As capital breeders, southern right whales (*Eubalaena australis*; SRWs) migrate from offshore summer foraging grounds to sheltered winter calving areas. Although aspects of female SRW reproductive behaviour have been studied at calving grounds through observation, information regarding female reproductive physiology is limited. In this study (approved by the University of Pretoria Animal Ethics Committee), endocrine profiles from baleen plates of four adult females that stranded on the South African coast between 1987 and 2013 are used to describe longitudinal patterns of female reproductive steroid hormones in this species for the first time. Subsamples from baleen plates taken at regular intervals were used for reproductive hormone (progestagens and oestrogens) and stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analysis. Baleen progestagen profiles showed pronounced, bimodal elevations above baseline at regular intervals interpreted as individual pregnancies separated by inter-calving intervals. Peaks in baleen oestrogen concentrations associated with elevated levels of progestagens were used to estimate time of parturition. Some progestagen profiles showed shorter intervals between peaks suggestive of either a loss of a pregnancy or a neonatal calf. From the generated profiles of this study, successful gestation was estimated to last approximately 15-18 months, which is somewhat longer than the 12-13 month gestation period estimated based on observational data. Most interestingly, from superimposing endocrine profiles with stable isotope profiles, we suspect that, in line with observational data, conception may not take place in coastal waters which has broader implications for connectivity between different SRW populations. These data provide novel information on SRW reproduction, critical in light of increased reproductive failure currently observed among the South African population.

New paradigm in tiger conservation: landscape-scale physiological correlates of various natural and anthropogenic factors across Terai-Arc landscape, India

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Abstract:
Tigers are among top ten large carnivores that has experienced substantial range contraction largely owing to human induced habitat loss. With decades of conservation efforts India has increased its tiger numbers however, in the face of continued habitat loss future challenge is to manage increasing tiger numbers within these available habitats. Knowledge of local and landscape scale factors that associate with biomarkers of physiological health of tiger can provide important direction to management efforts in making available and potential spaces more suitable. To this end, we collected faecal samples from all potential tiger habitats in the Terai-Arc landscape (TAL), a human dominated but global priority tiger conservation landscape holding 22% of India's current tiger population. Total of 345 fresh tiger confirmed faecal samples were used for hormone analysis. Change in faecal glucocorticoid (fGCM) and triiodothyronine (fT3M) metabolite measures were tested against key habitat features such as extent of human interference, forest cover type, prey occurrence (diet based), habitat connectivity and protection status. As this landscape contains one of highest tiger density areas in the world, local tiger density (intraspecies competition) was also included as a potential stress factor. We found that at local scale within protected areas, fT3M levels indicating nutritional status positively associated with increasing proportion of open forest area (including productive grasslands). fGCM levels indicating psychological stress associated positively with high local tiger density, suggesting an impact of intra-species competition pressure. Overall, across the landscape, increasing habitat connectivity along with increased forest cover and lower occurrence of livestock in diet associated with higher fT3M levels (improved nutritional status) in wild tigers. Results of this study provides evidence of poor health in tigers surviving in more disturbed and fragmented areas in this landscape. We emphasize on restoration of open forest systems and patch connectivity to improve overall habitat quality.

Longitudinal adrenal, reproductive, and thyroid hormone profiles from four 1960's bowhead whales

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Abstract:
Multi-year studies of individual mysticete whales are possible by utilizing the keratin matrix of baleen. As shown in previous work, steroid and thyroid hormones are deposited and stored along the length of each baleen plate. Once extracted, up to 20 years of hormone data are represented from a single individual that can reflect physiological responses to environmental and reproductive stressors. Corticosterone, cortisol, dehydroepiandrosterone (DHEA), progesterone, testosterone, and triiodothyronine (T3) have all been previously validated in bowhead whale (*Balaena mysticetus*) baleen. We created endocrine profiles for 2 adult males and 2 adult females from >50-year-old baleen. Hormone concentrations were quantified every 4 cm along the length of the plate (58-72 samples per individual) using commercially available enzyme immunoassays (Arbor Assays). Both females showed occasional dramatic elevations in progesterone, interpreted here as pregnancies, with the larger female showing repeated such cycles and the smaller female showing only one. DHEA, corticosterone, and testosterone also rose during these putative pregnancies. T3 and cortisol had marked variation, some of which may relate to annual cycles. Both males showed repeated testosterone elevations, likely annual cycles accompanied by simultaneous peaks in DHEA. The largest male showed a progressive decline in the amplitude of the testosterone and DHEA peaks, possibly related to his greater age. These data represent the first longitudinal endocrine profiles for these hormones in female bowheads, the first DHEA and T3 data for males, and add to our understanding of hormone patterns across multi-decade timespans in the longest-lived mammal. Our results underscore the importance of calculating baseline hormone concentrations based on life-history stage, the utility of historic specimens from natural history museums, and the value in evaluating several hormones concurrently in free-ranging wildlife studies.

Profiling the hidden whales of the Smithsonian: longitudinal hormone analyses of Antarctic baleen whales from the WWII era

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Abstract:
In 2017 the multi-year long effort to organize the Smithsonian Museum of Natural History's collection of over 1,800 Antarctic blue whale (*Balaenoptera musculus*) and fin whale (*Balaenoptera physalus*) baleen plates was completed. These plates were harvested over 70 years ago during the post-WWII era when Japanese whaling resumed. Because these plates grew over 4-7 years when there was no commercial whaling, minimal global climate change, and a hypothesized stable Antarctic prey base, these specimens may retain valuable information on baseline patterns of stress and reproductive hormones in large whales. We present results from longitudinal analyses of steroid hormones and bulk stable isotopes conducted on a sub-sample of the collection (n=18 plates: 4 males and 14 females). We quantified concentrations of reproductive steroid hormones (testosterone, progesterone) and glucocorticoids (cortisol, corticosterone) in both species and both sexes, from baleen powder sampled at 2-cm intervals along the length of each baleen plate. All specimens from females contained regions of sustained high progesterone, interpreted here as pregnancies. Apparent calving intervals ranged from 1-3 years, with pronounced individual variation. During putative pregnancies, both glucocorticoids were also elevated. In males, regularly spaced regions of high testosterone (putative annual breeding cycles) were present in some (but not all) individuals. Corticosterone was the dominant (more abundant) glucocorticoid in both species and sexes, with samples currently undergoing mass spectrometry quantification to confirm the ratio of metabolites. In conjunction with stable isotope analyses, these data assist our investigations of foraging ecology and breeding strategies of past baleen whale populations. These "baseline" hormone levels offer critical context for interpreting the calving intervals, breeding cycles, and stress physiology of modern day Antarctic blue and fin whale populations.

The lion's share of the lions' hair: insights into free-ranging lion stress and reproductive physiology using hair hormone analysis

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Abstract:
Human-carnivore conflict is a major cause for carnivore population declines globally which necessitates an increased understanding of anthropogenic impacts on wildlife physiology. Our goal was to determine if African lion (*Panthera leo*) hair hormone analysis can be used as a biomarker for stress and reproductive physiological changes in an area with varying degrees of human-lion conflict (HLC). Our objectives were to determine if there were relationships between 1) lions living in areas of higher HLC and glucocorticoid production, 2) lions in poor body condition compared to healthy lions, and 3) glucocorticoid and androgen production in males. We opportunistically collected hair samples from 2011 to 2022 (n=140) from free-ranging lions living in the Ngorongoro Conservation Area of Tanzania. We removed the follicles, washed and dried the hair, pulverized the hair, extracted the hormones using methanol, and analyzed corticosterone ("CC", Arbor Assays #K014) and testosterone ("T", males only, Arbor Assays #K032) concentrations. We measured HLC as the frequency of lion attacks on livestock within 30 km and 90 days of when the sample was collected. We did not find a relationship between higher rates of conflict and CC concentrations (R^2=0.02, df=138, P=0.11). We found that individuals that were in poor condition (mean: 31.0±5.3 ng/g) had higher (W=280, P=0.024) CC concentrations compared to those that were considered healthy (mean: 19.9±1.1 ng/g). There was a moderate correlation between male CC and T production (rho=0.48, P<0.001). Our initial findings indicate that lion hair hormone concentrations can demonstrate physiological changes, particularly for chronic stressors like poor body condition. We continue to evaluate this new data set to learn more about the relationships between free-ranging lions and environmental and anthropogenic stressors.

The cost of sympatry: spatio-temporal patterns in leopard dietary and physiological responses to tiger competition gradient in Rajaji Tiger Reserve, Uttarakhand, India

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Abstract:
Apex predators have critical roles in maintaining the structure of ecosystem functioning by controlling intraguild subordinate populations. Such dominant-subordinate interactions involve agonistic interactions including direct or indirect impacts on the subordinates. As these indirect effects are often mediated through physiological processes, it is important to quantify such responses to understand population parameters better. We used a large carnivore intraguild system involving tiger (*Panthera tigris*) and leopard (*Panthera pardus*) to understand the dietary and physiological responses under a spatio-temporal gradient of tiger competition pressures in Rajaji Tiger Reserve (RTR) between 2015-2020. RTR is a major source tiger population (estimated density of 8 ±1.4/100 km^2) and retains one of the highest densities of leopards (16.90 ±1.44/100 km^2) in the landscape. We conducted systematic faecal sampling in the winters of 2015 and 2020 from the park to assess diet and physiological measures from 5644 large carnivore faecal samples. Apart from competitive spatial exclusion, analyses of leopard-confirmed faeces (n=324) suggest a dietary-niche separation as a consequence of tiger competition. In 2020, we found an increased occurrence of large-bodied prey species in western-RTR (low tiger competition). Physiological measures followed the dietary responses where leopards with large-sized prey in the diet showed higher fT3M and lower fGCM measures in western-RTR. In contrast, eastern-RTR leopards showed lower levels of fT3M and fGCM in 2020, possibly due to intense competition from tigers. These patterns strongly indicate a physiological cost of sympatry where competition with dominant tigers resulted in elevated nutritional stress. The results highlight the importance of good-quality habitats and prey bases for the survival of this species. We recommend expansion of leopard monitoring and population estimation efforts to buffers, the developing appropriate plans for human-leopard conflict mitigation and intensive efforts to understand leopard population dynamics patterns to ensure their persistence during the ongoing Anthropocene.

Non-invasive quantification of reproductive hormone metabolites after a gonodotrophin-releasing hormone administration in the naked mole-rat (*Heterocephalus glaber*)

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Abstract:
The naked mole-rat (*Heterocephalus glaber*) is a subterranean mammal which occurs in colonies of up to 60 individuals. The colonies have a distinct dominance hierarchy whereby there is one dominant breeding female (queen), 1-3 breeding males, and the remainder of the colony are non-reproductive subordinates. The non-reproductive subordinates have reproductive capacity but are presumably reproductively suppressed by the queen. However, evidence thus far suggests that once removed from their natal colony, these non-reproductive subordinates are able to reproduce. To be able to non-invasively evaluate reproductive capacity in the species, we firstly had to examine the suitability of enzyme immunoassays (EIA) for determining progestagen and androgen concentrations in the naked mole-rat using feces as a hormone matrix. A saline control and gonadotrophin-releasing hormone (GnRH) were administered to twelve (six males and six females) naked mole-rats which were previously separated as dispersers based on their escape attempts from the natal colony. The results revealed that a 5α-Progesterone EIA was suitable for measuring fecal progesterone metabolite (fPM) concentrations in female naked mole-rats. In addition, an Epiandrosterone EIA has been identified to reliably measure fecal androgen metabolite (fAM) concentrations in both male and female naked mole-rats. Interestingly, preliminary results suggest that there is distinctive individual variation in baseline and peak fPM and fAM concentrations. Only two out of six females, and no males had an increase in fPM concentrations greater than 100% post GnRH administration. Conversely, only four out of six females, and three out of six males had an increase in fAM concentrations greater than 100% post GnRH administration. These preliminary results suggest that some naked mole-rat individuals potentially have a reduced reproductive capacity, even when separated from the queen. This may explain the individual variation in baseline reproductive hormone concentrations and why some individuals had no response to GnRH administration.

Characterization of basic reproductive biology in Amur leopards (*Panthera pardus orientalis*) using non-invasive monitoring

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Presentation type: Oral Presentation
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Abstract:
Amur leopards are classified by the IUCN as critically endangered with <60 individuals in the wild. In zoos, conservation efforts hinge on successful reproduction, and the AZA reports only 5.4 births per year. This leaves a 78% chance there will be no individuals left in zoos in less than 100 years. This study aimed to gain insight into their reproductive biology by examining estrus cycles, spontaneous ovulations, luteal phases, and pregnancies via fecal endocrine profiles. Five females in the continental U.S. were monitored from 2021-2023 (6-20 months): 2 were housed with a male, 2 were housed in the same building as a male, and 1 were housed singly. ELISA assays were conducted on fecal samples: progestogen metabolites (P4; n=4) to detect luteal phases and monitor pregnancies, and estradiol metabolites (E2; n=5) to detect cyclicity. Estrus was defined by 2 consecutive samples with E2 >1.5x baseline. Average length of estrus was 3.46(±0.47) days, inter-estrus interval was 11.16(±1.67) days. Mean E2 peak during estrus was 997.58 (±84.95) ng/g. A luteal phase was defined as 6 consecutive samples >2x baseline. In the two females that became pregnant, luteal phases lasted 53(±17.4) days. Spontaneous ovulation (SO) was observed five times over 12 months in 1 female housed without a male (no SO were observed in second female housed without a male). Average length of SO luteal phase was 26.8(±4.4) days. Mean peak P4 during pregnancy was 160,544.73 (±56,689.65) ng/g and mean peak P4 during SO was 103,990.51(±28585.35) ng/g. There was no significant difference between pregnancy and SO peak values ($p = 0.9214$). These data confirm that fecal hormones are an effective method to monitor female Amur leopards cyclicity and can aid in the application of assisted reproductive technologies.

How suppressed is suppressed? Long-term application of progestin as a contraceptive in giraffe

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Abstract:
Synthetic progestins have been used as a form of chemical contraceptive in many exotic and wild animal species, providing an effective and often essential tool for ex-situ breeding management; however, efficacy can be difficult to interpret as folliculogenesis and ovulation may persist, allowing observable oestrus behaviours to continue. This causes a particular challenge when managing mixed-sexed groups where persistent male interest may continue towards females despite contraceptive use; therefore, it is important to understand how suppressed hormone concentrations must be for contraception to be considered successful. To add further complication, progestins have been shown to cause weight gain and pathologies such as endometriosis in domestic horses so increasing doses to reach full suppression risks creating parallel challenges. Alternative contraception options, which require immobilisation or darting, may also present risks depending on temperament and management of such species. Following the daily administration of REGU-MATE® (altrenogest), an oral progestin solution, to five female Baringo giraffes (*Giraffa camelopardalis rothschildi*), faecal oestrogen (fE2; R4792) and progestagen (fP4; CL425) metabolite concentrations were paired with reports of male interest observed during routine husbandry. Altrenogest doses ranged from 0.047-0.2mg/kg per individual throughout the study and delivery methods varied (top-dressed or milled into feed) for four females on long-term contraception (duration: 4-7 years). A fifth female received top-dressed delivery only for an initial period of four months. Results show that during this initial (top-dressed) period, this female continued to receive male attention in line with cycling fP4 and fE2 with one full but unsuccessful mating seen three months into application. Conversely, sustained baseline progestagens were quickly observed from milled delivery but some male interest remained. Long-term use of altrenogest milled into feed reduces fP4 when compared with cycling concentrations but elevated concentrations and male interest occur intermittently; further exploration is needed to determine the cause of this variation.

Development of a 3D cell culture system to investigate mechanisms regulating corpus luteum functionality in felids

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Abstract:
Corpora lutea (CLs) are endocrine glands that play a crucial role in maintaining pregnancy. Understanding mechanisms regulating CL lifecycle in felids is needed to improve assisted reproductive techniques and to unravel functionality of persistent CLs in lynxes. In this study, we aimed to establish a cell culture system, which could be used as *in vitro* model for functional studies of CLs. We used samples from domestic cats (n=4) as a model species and later applied the methods to Persian (n=1) and Cloud (n=1) leopards (*Panthera pardus tulliana*, *Neofelis nebulosi*). Antral follicles were isolated from ovaries and digested with collagenase and DNase. The isolated mixture of cells was subjected for cell culture on ultra-low attachment plates to create spheroids; media composition should support luteinization. The cells were cultured for up to 23 days with periodic measurements of progesterone concentration by EIA. Gene expression of steroidogenic factors (*HSD3B1*, *STAR*, *CYP11A1*) and hormone receptors (*LHCGR*, *PRLR*, *PTGFR*) was additionally measured by real-time qPCR. From day 14, cells were treated with/without 1000 ng/mL cloprostenol (PGF2α-analogue) to validate the established cell culture model for functional studies. In the domestic cat, microscopic evaluation of cells indicated luteinization, which was confirmed by the significant increase of progesterone. The gene expression of *STAR*, *CYP11A1*, *LHCGR* and *PRLR* also significantly increased. Cloprostenol caused significant decrease in P4 secretion and downregulation of *STAR*, *LHCGR*, *PRLR* and *PTGFR*. Cells from Persian and Cloud leopards showed similar results for P4 secretion and gene expression as from domestic cats. In conclusion, our 3D cell culture system provides a valuable tool for investigating mechanisms regulating CL lifecycles, as luteinized cells maintained their steroidogenic activity for at least 23 days. The successful transfer of this model to cells of Persian and Cloud leopards suggests its potential in studying the functionality of CLs in wild felids.

Lipid content and hormones: how to get the most out of a single extraction

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Abstract:
Fecal samples are an accessible matrix for investigating physiological processes in endangered resident killer whale *(Orcinus orca)* populations. However, as sample volume is often limiting, it is important to find efficiencies in sample preparation to maximize opportunities for analysis. The goal of this study is to develop an extraction methodology that allows to measure both the lipid content and fecal hormone metabolites (FHM) from the same aliquots. Two protocols, (1) Folch and (2) Bligh and Dyer (B&D), using a solution of chloroform and methanol were used to extract aliquots from the same fecal samples. Lipid content was estimated gravimetrically from the chloroform residues and ranged from 5 to 26% and from 5 to 42% for Folch and B&D, respectively, with no difference between the two methods ($p = 0.29$). Methanol residues from both extractions were tested for suitability for FHM hormone analysis and compared to aliquots extracted in 70% ethanol, a solvent commonly used for this species and matrix. Specifically, these residues were validated and measured using enzyme immunosorbent assays for corticosterone and progesterone metabolites. FHM concentrations in methanol residues did not statistically differ between the Folch and B&D extractions ($p = 0.85$ for both corticosterone and progesterone metabolites). To compare FHM values between methanol and ethanol extractions, linear models were fitted to log-transformed concentrations, with values from Folch and B&D as the independent variable and those from ethanol extraction as the dependent variable. For both hormones, the model indicated a significant fit for Folch ($p < 0.001$, $R^2 = 0.8$ and $p < 0.001$, $R^2 = 0.9$ for corticosterone and progesterone metabolites, respectively) and B&D ($p < 0.001$, $R^2 = 0.8$ and $p < 0.001$, $R^2 = 0.9$ for corticosterone and progesterone metabolites, respectively). Results from this study showed that a single extraction can be used for multiple biomarkers, thus maximizing the use of small-volume samples.

Selecting your solvent: could it change your results?

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Abstract:
Faecal hormone monitoring provides a powerful, non-invasive tool for monitoring reproductive status and stress physiology in wild and captive animals. To investigate faecal hormone concentrations, the steroid metabolites must first be separated from the faecal material, which is typically done via an alcohol "extraction." Previous solvent comparison studies have identified the best solvent based on extraction efficiency. However, there is little information about how solvent type affects patterns of hormone expression or resulting conclusions. We compared three solvents (ethanol, methanol, and isopropanol) at two concentrations each (50% and 80%) and examined how solvent type affected both the measured hormone levels and expected patterns. Specifically, we compared differences between pregnant and non-pregnant samples across three species: Tasmanian devils (*Sarcophilus harrisii*), mice (*Mus musculus*), and giraffes (*Giraffa camelopardalis*). We measured progesterone, testosterone, and estradiol in all samples. Results varied across hormones: progesterone results were not significantly affected, testosterone results were slightly affected, and estradiol results were most affected by solvent type. Interestingly, the solvents that provided the greatest ability to distinguish pregnant and non-pregnant samples were not necessarily those that produced the highest overall concentration. These results highlight that researchers should consider how solvent type might affect biological trends, especially for estradiol.

An efficient way to evaluate multiple biomarkers in serum samples

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Presentation type: Oral Presentation
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Abstract:
It is an ongoing endeavor to find user-friendly methods for gleaning more physiological information from valuable wildlife samples. Whereas blood is generally preferred for biomarker analysis, the volume necessary for measuring numerous analytes can be difficult to obtain. Furthermore, conducting one assay for each analyte of interest is labor-intensive, often requires multiple freeze-thaw cycles, and generates much plastic waste. Luminex® offers an antibody-based system of detection that measures multiple analytes concurrently and requires small sample volume (<50µl in total). This system could alleviate volume constraints, provide potential time savings, mitigate freeze-thaw concerns, and reduce plastic waste. The objective of this study was to validate the Multispecies Hormone Magnetic Bead Panel (MSHMAG, EMDMillipore) for measuring testosterone, estradiol, progesterone, and cortisol in serum extracts from polar bears (acetonitrile [ACN]), and black and white rhinoceroses (ACN and diethyl ether [DE]). Extracts were run in duplicate according to kit instructions and measured on a MAGPIX® analyzer. Serially diluted pools of extracts (by species) were compared to the assay's standard curves. Spearman's correlation analyses were performed on results from rhinoceros serum extracted using both DE and ACN; multiplex results generated from rhinoceros DE extracts were compared to single hormone EIA values. In all three species, the multiplex concentrations for all four steroid hormones showed linearity in a dilution curve (R^2>0.9). Rhinoceros hormone values were positively correlated between extraction methods (R>0.86; P<0.011), except for estradiol (R=0.3; P=0.6). Extraction with ACN often resulted in estradiol values above expected physiological concentrations. Multiplex values for rhinoceros DE extracts were positively correlated (R>0.8; P<0.1) with the values from single hormone EIAs. In conclusion, the MSHMAG kit is a viable alternative for analyzing multiple hormones concurrently on serum from these species. This work was supported by grants from the Institute of Museum and Library Services MG-249011-OMS-21 and MA-249327-OMS-21.

Validating a fecal DNA damage assay via comparison to glucocorticoid metabolites in captive wildlife

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Abstract:
Reactive oxygen species (ROS) and other free radicals are by-products of cellular metabolism that can damage proteins, lipids, and DNA. An organism may experience oxidative stress when there is an increase in conditions that promote the formation of ROS, such as an increase in glucocorticoids (GCs). Recent studies in conservation physiology have employed biomarkers of oxidative status to understand how free ranging animals are physiologically impacted by environmental stressors, suggesting that applying these measures to captive wildlife could be informative about their welfare. We aimed to validate an assay for fecal DNA damage by examining its relationship to fecal glucocorticoid metabolites (FGMs) in five species at the Detroit Zoo. We collected samples from N=2 male white rhinoceros (*Ceratotherium simum*) throughout a COVID-19 closure, N=4 female chimpanzees (*Pan troglodytes*) and N=3 male western lowland gorillas (*Gorilla gorilla gorilla*) surrounding veterinary immobilizations, N=1 female polar bear (*Ursus maritimus*) during a conspecific move, and N=3 male grizzly bears (*Ursus arctos horribilis*) throughout habitat modification and veterinary immobilizations. We tested fecal glucocorticoid metabolites (FGMs) using an in-house corticosterone enzyme-immunoassay (CMJ006, C. Munro) or a cortisol enzyme-immunoassay (ISWE002, Arbor Assays) validated via parallelism and recoveries for each species. We tested for DNA damage using an enzyme-immunoassay (K059, Arbor Assays) which targets 8-hydroxy-2'-deoxyguanosine (8-OHdG) validated in fecal extracts for each species via parallelism and recoveries. Regression results show that there was a significant positive relationship between log(8-OHdG) and log(FGM) concentrations for the grizzly bears ($F(1,36)=6.55$, $p=0.015$), the polar bear ($F(1,13)=30.77$, $p<0.001$), one of the male gorillas ($F=(1,11)=15.38$, $p=0.002$), and the chimpanzees ($F(1,32)=24.38$, $p=0.001$). There was a negative correlation between log(8-OHdG) and log(FGM) for one rhinoceros ($r=-0.39$, $p=0.02$). These results validate our ability to measure by-products of DNA damage in fecal samples, and suggest there is a relationship between adrenal activity and DNA damage.

Does wildlife friendly lighting have a physiological cost? Investigating the impacts of LED lighting on melatonin and glucocorticoid expression in nocturnal mammals

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Abstract:
Artificial light at night is one of the fastest growing forms of urban pollution and has been identified as a key threat to biodiversity. Artificial light fundamentally changes the night-time environment by masking natural light cues and desynchronising the internal clock. Energy-efficient lighting, such as white light emitting diodes (LEDs) may have an even greater impact on wildlife. This is because white LEDs consist primarily of short (blue) wavelengths, and these wavelengths play a greater role in regulating circadian rhythms. However, one advantage of LED light sources compared to traditional lighting is the flexibility to change the wavelength composition. We examined the effect of short-wavelength white LED lighting (standard urban lighting) and long-wavelength amber LEDs (proposed wildlife friendly lighting) on urinary melatonin and faecal glucocorticoid metabolites in Krefft's gliders (*Petaurus notatus*) and Gould's wattled bats (*Chalinolobus gouldii*). Melatonin and glucocorticoid secretion typically follow a circadian pattern of release regulated by natural light-dark cycles. We found that exposure to short-wavelength white LEDs disrupted normal melatonin and glucocorticoid expression in Krefft's gliders. In contrast to previous studies, exposure to long-wavelength amber LEDs also disrupted melatonin and glucocorticoids. Conversely, we did not detect any disruption to urinary melatonin or glucocorticoid expression in Gould's wattled bats, irrespective of light spectra. We provide evidence that long-wavelength LEDs can disrupt physiology and may not be a wildlife sensitive lighting option for all species. Our findings highlight the importance of developing species-specific knowledge of the effects of artificial light at night on wildlife physiology. Findings from these studies will inform management of artificial light at night and support the development of wildlife friendly lighting.

The role of mesotocin in regulating social behavior in Siberian jays: insights from a wild population

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Abstract:
Although we have a good understanding of the evolutionary drivers of cooperation, our understanding of the proximate mechanisms that govern cooperation is limited. In vertebrates, hormones from the vasopressin family (oxytocin, mesotocin) are linked to the expression of social behaviors such as gregariousness and pair bonding. However, due to its low peripheral concentrations, the effect of these hormones on wild populations remains unexplored. Here, we conducted a study using a combination of mesotocin titers in blood plasma and mesotocin intranasal administration to investigate how mesotocin affects and predicts social behavior in a wild population of Siberian jays. This species live in stable groups consisting of a breeding pair and up to three kin and/or non-kin non-breeders. Repeated sampling showed that individuals with higher mesotocin levels tend to show a greater inclination to stay with unrelated group members. These results were further supported by experimental mesotocin administration. Overall, this study provides evidence for the critical role of mesotocin in regulating social behavior in birds.

Alterations in faecal glucocorticoid metabolite concentrations post-defaecation across three animal feeding classes (ruminants, hindgut fermenters and carnivores)

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Abstract:
Quantification of faecal glucocorticoid metabolites (fGCMs) is a popular non-invasive technique for monitoring wildlife's reactions to stressors. To ensure comparability of determined values for a studied species, information regarding the duration for which fGCM composition post-defaecation remains stable is essential to pinpoint the time period that a sample can be collected post-production. In the present study, we measured the rate at which fGCM concentrations of nine different species belonging to three animal feeding classes' (ruminants, hindgut fermenters, and carnivores) change throughout a 7-day period post-defaecation. Enzyme immunoassays previously established for the respective species in question were used for fGCM quantification in giraffe (*Giraffa camelopardalis*), impala (*Aepyceros melampus*), blue wildebeest (*Connochaetes taurinus*), plains zebra (*Equus quagga*), African elephant (*Loxodonta Africana*), white rhino (*Ceratherium simum)*, cheetah (*Acinonyx jubatus*), spotted hyena (*Crocuta crocuta*), and leopard (*Panthera pardus*). The findings demonstrate a significant decrease in fGCM concentrations for all herbivorous species ranging from 6h (Impala) to 48h (giraffe, blue wildebeest, white Rhino, and African elephant) post-defaecation. For carnivores, monitored fGCM concentrations remained comparable for seven days for cheetah and leopard, but started to increase after 24h in spotted hyenas, before dropping to comparable levels again after 48h. Given that logistical challenges often restrict the frequent collection of faeces for endocrine monitoring in free-roaming wildlife, this study provides the first structural indication regarding the time frame during which faecal sampling should be scheduled for species belonging to a certain feeding class. However, alterations in fGCM concentrations post-defecation still have to be determined as a quality measure for a species investigated for the first time, or when utilizing a new quantification protocol.

An attempt to evaluate the physiology of wild snow leopards using fecal steroid hormone analysis

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Abstract:
Hormones can provide insights into the hidden physiological states of animals and the impacts of their surrounding environment, including stress responses, which have recently gained attention in conservation biology. In this study, we attempted to understand the stress level of wild snow leopards using fecal samples and whether stress level is correlated with human density. First, we standardized fecal hormone analysis methods using fecal samples of captive snow leopards and then applied the standardized protocol to the 90 fecal samples of wild snow leopards collected in Shey-Phokusundo National Park, Nepal during the breeding season (March-May 2021). We specifically compared fecal glucocorticoid and sex steroid hormone metabolite concentrations among four villages that differed in prey animal density, livestock population, and human population. Preliminary results revealed that snow leopard fecal samples collected around the heavily populated human settlements tended to exhibit higher glucocorticoid levels. Additionally, some female samples showed high concentrations of estradiol-17β, progesterone and/or 13,14-dihydro-15-keto-PGF2α, indicating that certain individuals were estrus, pregnant or pseudopregnant. Snow leopards often attack livestock and lose their lives due to retaliatory killing by herders. Conflict with humans over livestock depredation has become a major threat to this endangered species. However, the cause of snow leopard attacks on livestock is unknown. For example, pregnant females may be more inclined to attack livestock that are easier to target due to their increased energy needs. If the information on stress, estrus, and pregnancy status can be obtained from snow leopard feces collected around villages, we can better understand the physiological characteristics, including stress level, of individuals that engage in livestock attacks. Overall, the preliminary findings of our study highlight the potential of using non-invasively collected fecal samples to understand the anthropogenic impact on species' internal physiological state.

Expression of glucocorticoids across the ovarian cycle in the critically endangered mountain pygmy-possum

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Presentation type: Oral Presentation
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Abstract:
Studies of female reproductive endocrinology typically focus on estradiol and progesterone. However, there is growing recognition that glucocorticoids also play an important role in promoting reproductive function. The goal of this study was to examine patterns of GC expression across the ovarian cycle in the critically endangered mountain pygmy-possum (*Burramys parvus*). Mountain pygmy-possums are a unique Australian marsupial that inhabit alpine habitats. Their conservation depends on the success of captive breeding programs, which in turn rely on knowledge about reproductive endocrinology. We monitored urinary GC concentrations (corrected for creatinine concentration) in 8 females throughout the reproductive season. We found that glucocorticoids fluctuate in a predictable manner across the ovarian cycle, with peak GC levels occurring during the follicular phase. Recognising the role of GCs in ovarian function will not only yield new strategies to diagnose and treat reproductive issues, but will also improve our ability to understand how other disruptions to GC expression (stress, artificial light at night) might impact reproduction.

Adreno-immunological response in rehabilitation processes of ex-situ in tropical screech owl (*Megascops choliba*)

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Abstract:
To prevent wild species trade, each country's governmental entities have rescued-specimen attention and rehabilitation centers. However, most of these specialized centers only provide ex-situ care, generating challenging situations that can compromise the patients' health. This study aimed to evaluate the endocrine and immunologic parameters of (*Megascops choliba*) owls along 49 consecutive days in captivity. Twelve adult owls were exposed to reproduction of audios recorded in the hospital setting of a rescue center between days 15 and 35. Fecal samples were taken every seven days, as were blood samples from eight of these animals. Plasmatic level of corticosterone and its metabolites (MGC) was quantified by enzymatic immunoassay. Leucocyte profile and plasmatic bactericidal competence (PBC) to counter *Escherichia coli* were evaluated. Two out of eight owls showed peak corticosterone in plasma values during exposure to acoustic treatment. The highest MGC concentration in the samples taken during acoustic treatment was statistically confirmed, independently of gender. The highest PBC values were registered on the day before stimulus, as compared with the values registered on the days with or after stimulus. No significant difference was established in cell Hemogram (neutrophils and lymphocytes). The results indicated that exposure to hospital-setting noise generated physiological changes at an individual level.

Physiologically plastic elephants: highly dynamic glucocorticoid responses in African and Asian elephants

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Abstract:
Recent studies show that elephants are behaviourally 'adjusting' in relation to changing environments and contexts. Such flexibility may be driven by the underlying physiological mechanisms. Assessing how behavioural and physiological adjustments influence the survival and fitness of long-lived, slowly reproducing elephants and whether the favourable traits get expressed over generations becomes crucial. In addition, anthropogenic challenges further may influence these flexibilities in elephants. One of the predominant proxies of physiological stress, glucocorticoids (GC) has been used widely to assess the physiological state of elephants across different contexts. We reviewed published findings on stress physiology of elephants, from 1980 to 2022, and documented that stress responses, as measured by GC in different sample matrices, often are highly dynamic and vary within and across individuals exposed to similar stimuli, and not always in a predictable fashion. Such dynamicity in physiological reactivity may be mediated by differences in personality traits or coping styles, or ecological conditions, and even technical factors that often are not considered in study designs. We introduced 'Physiological Dynamicity Model' to explain the influences of contexts, experience and personality in how elephants perceive the challenges and how that influence the stress reactivity. We emphasize on (i) cautious interpretations of GC data in the context of normal adaptive stress versus distress and (ii) the need for long-term studies on GC concentrations, including other markers of stress, to determine the fitness consequences of physiological plasticity in elephants.

Development and validation of a steroidomics methodology for non-invasive biomonitoring in wildlife

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Abstract:
Wildlife conservation is often challenged by lacking knowledge about the reproduction and adaptability of endangered species. For giant pandas, as an example, the (patho)physiological processes during pregnancy are still largely unknown, which complicates their breeding and conservation programs. Although monitoring steroids and related molecules could increase this knowledge, current techniques (e.g. immunoassays) are hampered by the species-specific steroid metabolism and the obligatory use of non-invasive samples. Therefore, this study aimed to develop a 'steroidomics' method for (un)targeted screening of a wide range of sex and stress steroids and related molecules in urine using UHPLC-Q-Orbitrap-HRMS. The method was optimized using spiked pooled giant panda urine samples. In total, 50 steroids (conjugated and non-conjugated androgens, estrogens, progestogens and glucocorticoids) and 6 prostaglandins standards could be uniquely detected based on *m/z* ratio and retention time. For the validation, ammonium acetate buffer solution was used as blank matrix. A total of 52 out of 56 standards demonstrated a detection limit below 0.05 ng/µL. Excellent linearity (R^2>0.99), precision (CV<20%), and recovery (80-120%) were observed for 46, 46, and 39 standards, respectively. Untargeted screening of pooled giant panda samples yielded 9624 components with CV<30% from which 78.4% also demonstrated excellent linearity (R^2>0.90). The method was applied to male and female giant panda urine, as well as pooled human samples. A total of 29 different steroids could be detected

with clear qualitative and quantitative differences between human and giant panda samples with human urine containing higher levels of glucocorticoids and 11-ketoetiocholanolone glucuronide as most abundant androgen, compared to b-nortestosterone in male giant panda samples. Remarkably, female giant panda samples from the late luteal phase also contained high levels of b-nortestosterone, together with allopregnanolone sulphate and prostaglandin D2, while oestrus samples contained high levels of oestrone. These results demonstrate the analytical and biological validity of our steroidomics method.

Preliminary use of urinary NIRS for early pregnancy detection in giant pandas

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Abstract:
Detection of giant panda pregnancy is currently limited to the final two to three weeks of the luteal phase based on ultrasound and urinary hormone measurements with an earlier pregnancy test elusive. Near infrared spectroscopy (NIRS) has been utilised successfully in non-invasive disease detection in a range of species, and estrus detection in giant pandas. We aimed to pilot the potential of NIRS for giant panda luteal phase monitoring and pregnancy detection. Urine from the final 50 days of five luteal phases of three females was assessed for NIRS spectra alongside five typical analytes included in giant panda reproductive monitoring (creatinine, urinary specific gravity, estrogens, progestogens and PGFM). These five luteal phases included one pregnancy, two pseudopregnancies and two non-birth cycles. Modelling from the NIRS spectra resulted in successful prediction of all five reproductive analytes. NIRS spectra were then used to develop aquagrams for pregnancy and pseudopregnancy, with visual differences apparent across the whole final 50 days of the luteal phase. Non-birth aquagrams were similar to pregnancy, however deviations were present in the final 30 days of the cycle. Concluding, NIRS has potential for further investigation as an early rapid, non-invasive and non-sample-destructive pregnancy test in giant pandas.

Record-breaking mortality of Florida manatees: can fecal hormone assessment help provide early warnings of population consequences?

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Presentation type: Oral Presentation
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Abstract:
Over the past two decades, nine mass die-offs of Florida manatees (*Trichechus manatus latirostris*) have been documented, resulting in the deaths of more than 2,000 animals. Currently, an ongoing *Unusual Mortality Event* (UME) along the Atlantic coast of Florida, USA, has resulted in a record number of manatee deaths, with 582 carcasses found during a 4-month period (Dec 2020–Mar 2021). The ability to monitor the physiological responses of manatees to threats and identify changes, potentially *before* deleterious population consequences are observed, could greatly enhance management and mitigation efforts. Health assessments of wild manatees using capture techniques provide an enormous amount of valuable data; however, there remains a need for a noninvasive and practical tool to monitor and detect changes in manatee health at the population level (increasing sample sizes in a cost-effective way). Here, we validated fecal enzyme immunoassays to monitor manatee health by measuring reproductive activity (progesterone and testosterone), adrenal stress (cortisol) and metabolic thyroid function (triiodothyronine, T3) using feces collected from manatees during live health assessments (n = 132) and post-mortem investigations (n = 101). Apparently healthy manatees had elevated fecal cortisol and T3 during spring months likely related to heightened metabolic demands due to breeding activity and increased food availability. Fecal cortisol and T3 levels were lower in winter, when food becomes scarcer, and manatees possibly conserve their energy reserves. Meaningful patterns in stress and metabolic hormones related to acute and chronic stressors persisted in samples despite the potential for bacterial degradation of hormone metabolites in dead manatees. Manatees that died during two different UMEs (2013 and 2021) showed significant adrenal and thyroid activation compared to healthy manatees. This non-invasive tool could be useful to enhance manatee health monitoring during human-caused and environmental perturbations, which are increasing in frequency and often challenging to investigate.

All eggs in one poor quality basket: assessing Hawaiian green sea turtle (honu) resilience in light of climate change impacts

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Presentation type: Oral Presentation
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Abstract:
The Hawaiian green sea turtle (honu) primarily reproduces at one small and remote atoll known as Lalo. Historical evidence suggests most honu make a reproductive migration to Lalo from the main Hawaiian Islands (MHI) to nest and bask on multiple islets within the atoll. However, Lalo is low-lying, making it particularly susceptible to climate impacts. From 1963–2018, two critical nesting islets were completely lost due to natural erosion while one was reduced to half its size by a hurricane (East Island). Tern Island is the only remaining reliable landmass for egg incubation where nesting habitat is not dynamic throughout the season. To assess honu's resilience to the drastic change of their primary nesting beach (East Island), we hatched a plan to determine whether females would continue to lay their eggs in one (poor quality) 'basket'. Firstly, adult females that previously nested on East Island were captured while basking on beaches within the MHI, and their reproductive status was assessed via (1) a portable ultrasound device to visualize ovarian follicles and (2) testosterone concentration. In 2021 and 2022, four females were satellite tagged prior to their reproductive migration from the MHI. Secondly, eight females that nested on East Island during previous nesting seasons were satellite tagged while basking there prior to the peak of the nesting season (during 2021 or 2022) to assess nesting beach fidelity after arriving at Lalo. This culminated in 12 satellite tagged females; five females only nested on East Island, three used both East Island and other islets (including Tern Island), and four turtles nested only on Tern Island. Thus, these findings suggest honu are capable of using multiple nesting habitats and provide hope for honu resiliency. Our study which used reproductive biology techniques has significant implications for the conservation of this threatened sea turtle population.

Faecal androgen metabolites exhibit predictable fluctuations across the ovarian cycle in Tasmanian devils (*Sarcophilus harrisii*).

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Presentation type: Oral Presentation
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Abstract:
Understanding a species' reproductive physiology is crucial to successful captive breeding. The Tasmanian devil (*Sarcophilus harrisii*) is an endangered marsupial for whom captive breeding is beneficial but challenging, as their cryptic nature necessitates a non-invasive approach to reproductive management. Prior Tasmanian devil studies have mapped patterns of oestradiol and progesterone across the ovarian cycle. However, androgens have not yet been explored in the female devil. There is growing recognition that androgens play an important role in female reproductive development and function, and can be an accurate predictor of reproductive stage. This study examined patterns of faecal androgen metabolites (FAM) during the ovarian cycle of the female Tasmanian devil. Faecal samples were collected daily from seven captive female devils (10 oestrous cycles) and analysed for FAM via enzyme-immunoassay. Cycles were aligned using characteristic changes in progesterone metabolites, and peaks were classified as 2SD above baseline. We observed a bimodal peak in FAM, starting 26 days before parturition and lasting for ten days before returning to baseline. This increase aligns with the timing of oestrus in devils. There was also a large, one-day spike in FAM at parturition in all but one cycle. This study is the first to explore patterns of FAM throughout the female devil's reproductive cycle. This improved understanding of the reproductive physiology in the Tasmanian devil will thereby provide another tool to support captive breeding efforts.

Characterization of 13,14-dihydro-15-keto-prostaglandin f2-alpha metabolite (PGFM) in the sand cat (*Felis margarita*) and Pallas' cat (*Otocolobus manul*) as a non-invasive marker for pregnancy resulting from natural breeding or artificial insemination

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Presentation type: Oral Presentation
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Abstract:
In felids, the increase in progesterone during a non-pregnant luteal phase (NPLP) is indistinguishable from pregnancy, and NPLPs can last up to two-thirds the length of gestation. One promising pregnancy marker is 13,14-dihydro-15-keto-prostaglandin-F2alpha-metabolite (PGFM), a stable fecal metabolite of prostaglandin F2alpha. Our objectives were to characterize fecal PGFM during NPLP and pregnancy in sand cats (Scats) and Pallas' cats (Pcats) using a commercially-available enzyme-linked-immunoassay. PGFM was assessed in eight Scats (n=3 natural breeding (NB) pregnant; n=1 AI pregnant; n=2 NB non-pregnant, n=2 AI non-pregnant) and ten Pcats (n=3 NB pregnant; n=2 AI pregnant; n=3 NB non-pregnant, n=2 AI non-pregnant). In Scats, baseline PGFM was different between individual cats ($P<0.0018$) but was not different in the same cat between different years. In Pcats, baseline PGFM was different between cats ($P<0.0001$) and between different years in the same cat ($P<0.0001$). "Cut-off" was calculated as the maximum PGFM value observed in any non-pregnant cat plus two SDs of PGFM among all non-pregnant cats. Two different criteria were used for anchors: day after which the individual had no more false negatives, and day of first positive sample. PGFM crossed the cut-off in 50% of pregnant individuals at day 36 in Scats and day 40 in Pcats. No false negatives are observed after day 51 in Scats and day 61 in Pcats. There is no significant difference for the day of pregnancy detection between AI and naturally pregnant cats, for either anchor. PGFM rises in all pregnant Scats as gestation progresses ($P=0.0208$) and is unaffected by the number of kittens. PGFM rises in all pregnant Pcats as gestation progresses ($P<0.0001$); there is a significantly greater rise in PGFM concentrations in late gestation in females carrying more kittens ($P=0.0459$). These data demonstrate the utility of PGFM as a diagnostic indicator of pregnancy in Scats and Pcats.

Non-invasive reproductive monitoring helps in successful breeding of mouse deer (*Moschiola indica*) in captivity

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Presentation type: Oral Presentation
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Abstract:
Mouse deer (*Moschiola indica*) plays a major role in the forest ecosystem as a seed disperser and forms an important prey for small and large carnivores; it is endangered because of extensive poaching in India. Despite its importance, no information on breeding and the reproductive physiology of this species was available. As part of the conservation breeding program, we recorded observations on breeding characteristics and discovered a unique postpartum estrus whereby mating was observed within 4-6 hrs. following the parturition. The present study aimed to develop and standardize enzyme immunoassays for monitoring the postpartum estrus and reproductive physiology using faecal progestagen and estrogen in female mouse deer. High-pressure liquid chromatography (HPLC) analysis revealed immunoreactive progestagen and estrogen in faecal samples. A total of 900 faecal samples were collected from 10 adult captive female mouse deer over one year from Nehru Zoological Park, Hyderabad, India. Postpartum estrus was observed after parturition, as evidenced by estrogen peaks during 2-3 days before and after delivery and subsequently returned to the baseline. The mean (±SEM) concentration of faecal estrogen was 298.5 ± 76.6 ng/g, 2-4 days before and 228 ± 30.7 ng/g, 2-3 days after parturition. The faecal progestogens concentrations were significantly declining on the day of parturition and mating shows effective stimulant for estrus behaviour in mouse deer. The mean (±SEM) concentration of faecal progesterone metabolites was significantly elevated in pregnant (41254.7 ± 1497 ng/g) compared to non-pregnant animals (11277 ± 745 ng/g) ($P<0.001$). Mounting, mating, and postpartum estrus were observed immediately following the delivery of the fawn and recorded as unusual behaviors among the mammals. Our findings directly helped in the successful breeding and managing of the mouse deer in captivity from 8 to 450 individuals and about 250 individuals were successfully introduced into the wild.

Validation of non-invasive hormone analysis techniques to assist in the identification of maternal roosts of ghost bats (*Macroderma gigas*)

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Presentation type: Oral Presentation
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Abstract:
The ghost bat (*Macroderma gigas*) is exclusively nocturnal, and the largest (~100 g) and only carnivorous microbat in Australia. They occupy caves, disused mine adits and rock crevices as daytime roosts but distinguishing which are preferred as maternal roosts is challenging as bats are easily disturbed while roosting. Identification of maternal roosts is a priority for conservation and management purposes, and therefore non-invasive hormone analysis was investigated as a potential tool to increase our understanding of ghost bat reproductive biology and identify the cave preferences of pregnant individuals. To validate these techniques, fecal samples were collected from group housed female ghost bats at Perth Zoo between October and January, during the expected parturition period in 2015, 2016 and 2017. Fecal samples were weighted (0.025 g), extracted with 2.5mls of 80% methanol and analyzed for progesterone metabolite levels (FPM) by enzyme-immunoassay (Coralie Munro, UC Davis, USA). Over three years, four pups were born with birth dates ranging from early November to late December suggesting a minimum gestation length of at least 120-172 days, with evidence of variability. The mean FPM levels were lower in non-pregnant females than in pregnant females (206.5 ± 102.9 ng/g and 7003.6 ± 6078.0 ng/g respectively). As collection mats are placed in caves for one to two months for *in situ* fecal collection, a hormone degradation study was also undertaken. Degradation tests were performed in the lab under two different temperature and humidity combinations for 6 to 8 weeks, to mimic possible cave conditions There was no change in classification of samples (baseline vs pregnant) over time for either set of environmental conditions. This data will provide valuable information for the conservation and management of the ghost bat and has the potential to be applied to other bat species.

Utilising routine non-invasive faecal samples for the detection of oestrus and early gestation in okapi (*Okapi johnstoni*)

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Presentation type: Poster
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Abstract:
Okapi (*Okapi johnstoni*) are one of the few surviving species within the family giraffidae and were classified as endangered by the IUCN (International Union of Conservation and Nature) in 2013 due to continual population decline. To assist in the species' survival, sustainable *ex-situ* populations are required, and therefore understanding the reproductive status of individuals is important for population management. In this study, the oestrous cycles and gestations of four okapi (n=7 pregnancies) housed at two EAZA (European Association of Zoos and Aquariums) institutions, were monitored through non-invasive faecal sampling methods with hormone metabolites measured using biochemically validated progesterone (CL425) and estrone-conjugate (R522-2) enzyme immunoassays. Baseline criteria for determining oestrous cycles was developed using approximately 20 months of oestrous cycles from each of two females, before being applied to pre- and early conception data. Pre-conception oestrous cycles were characterised with luteal phase (n=10) of 8.10 ± 0.94 days and an inter-luteal phase (n=9) of 7.00 ±0.67 days. Oestrogen metabolite peaks occurred at the beginning of the inter-luteal phase with peaks every 14.78 ± 0.84 days as progestagen metabolites were decreasing to baseline. Gestation was confirmed when progestagen metabolite concentrations remained at luteal phase concentrations for at least 20 days, with concentrations remaining at this level for the first 120 days of gestation without return to baseline values following mating. A secondary increase at around 150 days lasted until parturition. This data demonstrates that okapi exhibit similar oestrous cycles to other members of the giraffidae family, with faecal oestrogens for the first time indicating that ovulation occurs at the beginning of the inter-luteal phase while progestagens are still elevated. Furthermore, we have shown that through routine sample collection and hormone monitoring, gestation can be confirmed within the first trimester, which can benefit the management of the species in *ex-situ* populations.

Successful pairing, monitoring, and pregnancy detection of large spotted genet (*Genetta tigrina*)

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Presentation type: Poster
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Abstract:
Non-Invasive monitoring of the female reproductive cycle is an important tool to determine reproductive status and time introduction of pairs, however the female reproductive cycle remains unmapped for many species. In November of 2018, we began monitoring one female large spotted genet (Genetta tigrina) housed alone to determine if we could use hormonal patterns to provide optimal timing to introduce a male. Previous attempts resulted in injury of the male. Other than approximate gestation length and seasonality of breeding and births, no additional information on the spotted genet reproductive cycle is available. Urine was collected daily from November 2018 through April 2022. We measured progesterone (CL425) and estrone-3-glucuronide (R522–2) to determine if either hormone could give insight to the genet reproductive cycle. Over the first year of monitoring that the two hormones fluctuated similarly and therefore continued to monitor urinary progesterone. While housed alone, urinary progesterone and estrogen cycled up and down over a 9–13-day period with 4-6 days elevated and 4-6 days at baseline (short cycle) or exhibited extended periods of baseline anestrous. The pair was successfully introduced in November of 2019, the female than exhibited longer cycles associated with mating in addition to short cycles and periods of anestrous. Long cycles, possibly lost pregnancies or pseudopregnancy, averaged 36.8 ± 3.8 days with average peak progesterone 24.0 ± 9.8 ng/mgCr while short cycles lasted 11.8 ± 3.5 days and peaked to 28.7 ± 9.8 ng/mgCr. Pregnancy was diagnosed when progesterone remained elevated for 45 days and a nest box and cameras were placed. Gestation lasted 71 days and parturition was marked with a rapid decline to baseline. Three years of monitoring allowed us to successfully pair incompatible animals, determine pregnancy, and intervene with lifesaving medical care when the female presented with dystocia, and suggests that this species exhibits a unique reproductive cycle.

Longitudinal analysis of reproductive and adrenal hormones in Bontebok (*Damaliscus pygargus*): a case study of social changes and reproductive events

Candace Scarlata[1], Kelly Gomez[2], Amy Hash[2], Michelle Shireman[2], Michael Suhn[2], Anne Lauerman[2], Beth Foster[2], Virginia Grimley[2], Laurel Fink[1], Nadja Wielebnowski[1]

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Abstract:
Bontebok (*Damaliscus pygargus*) are one of the rarest antelope species in South Africa and a great example of how conservation was able to bring a species back from the brink of extinction. Surprisingly, given long-term conservation efforts, relatively little is known about their physiology. In 2016, the Oregon Zoo received a pair of male bontebok, but excessive sparring led to re-location of one male and the acquisition of a female. As part of our animal welfare program, fecal samples have been collected continuously 2-3 times a week since the species' arrival. The main objectives of our monitoring efforts were to: 1) Validate fecal adrenal and reproductive hormone metabolite enzymeimmunoassays (EIAs) for male and female bontebok; 2) Monitor variations in adrenal hormones during different veterinary procedures and 3) Characterize hormonal changes associated with social interactions and reproduction. Using extracts of fecal metabolites, we successfully validated EIA assays for this species for three hormones: glucocorticoids (fGM), progestagens (fPM), and testosterone (fTM). Biological validation of our fGM assay was provided by observing significantly elevated fGMs the day after a veterinary procedure. In addition, intermale aggression resulted in elevated fGM concentrations in the more subordinate male. Validation of our fPM assay was provided by the monitoring of estrous cycles and pregnancy in the female (fPM). Estrous cycles lasted 20-26 days (21.86 ± 0.74 days). Four pregnancies were monitored in the same female and were characterized by elevated fPMs for 224-229 days (226 ± 1.22). The male showed seasonal changes in testosterone with increased fTM concentrations from May to September. Interestingly, bontebok breeding season in South Africa is reported to last from January to March, but in our bontebok this season is shifted 6 months and lasts longer. Ultimately, we hope that these data can provide valuable physiological information for future breeding and conservation efforts.

Assessing reproductive hormones in adult female blue whales (*Balaenoptera musculus*) by analyzing historic baleen samples from the 1940's

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Abstract:
Monitoring reproductive parameters such as gestation period and calving intervals in blue whale (*Balaenoptera musculus*) populations is difficult due to limitations in hormone data collection methods for large whales. The most significant hindrance is that it is impossible to collect blood samples from these animals due to their size; therefore, alternative methods for hormone analysis must be investigated. Recent studies have shown that hormones like progesterone can be detected in baleen powder of other mysticete whale species like bowhead (*Balaena mysticetus)* and North Atlantic right (*Eubalaena glacialis)* whales. I hypothesized that baleen from female blue whales would contain regions of high progesterone indicating prior pregnancies. To test this, I ran enzyme immunoassays to quantify progesterone from serial samples taken along the length of historic baleen plates of four female blue whales, two of which were known to be pregnant upon capture. All of the specimens in this study were originally captured by post World War II era Japanese commercial whaling vessels and are archived at the Smithsonian National Museum of Natural History. This time period overlaps a pause in commercial whaling globally, and predates significant impacts of climate change, implying a relatively low stress environment for this population compared to recent years. Most baleen plates had several broad regions of high progesterone, as predicted, with patterns suggesting a 2-year calving interval for all but one female. A possible reason for this outlier could be due to the age of the individual, as she was the smallest individual in the sample size and may have not yet been reproducing. Two individuals known to be pregnant at death had high progesterone in the most recently grown baleen, as predicted. These findings may clarify historic norms of reproduction in blue whales, and could be helpful for comparisons to modern populations.

Calving intervalsiInferred from progesterone patterns in historic baleen of female fin whales (*Balaenoptera physalus*)

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Abstract:
Reproductive patterns were once a cryptic topic in whale physiology due to the inaccessibility of obtaining endocrine data from mysticete whales. However, recent advanced approaches in measuring hormones in baleen reveal the possibility of obtaining multi-year reproductive hormone profiles from various species of mysticete whales. Hormone analysis of baleen thus offers a tool to evaluate how ecological and anthropogenic pressures impact whale physiology. We investigated progesterone patterns in historic WWII-era baleen of four female fin whales (Balaenoptera physalus) to (1) develop the first longitudinal hormone profiles of fin whales and (2) evaluate gestation periods and calving intervals in a time period when global climate change was minimal and there was a cessation of commercial whaling. Historic baleen plates from the Smithsonian National Museum of Natural History were drilled every other centimeter to obtain a pulverized powder for hormone extraction. Enzyme immunoassays were run to measure progesterone concentrations of each whale (4 females, pregnant at the time of capture) over ~3-4 years. Results indicate a likely one- to two-year calving interval in fin whales, but with notable individual variation. This pilot study helps provide a baseline of female fin whale progesterone patterns, thus offering data that can be compared to modern-day whales that are subjected to the effects of climate change. Future research can assess the influence of modern anthropogenic stressors by identifying abnormal reproductive patterns which can influence recovery efforts and management strategies.

Changes in testicular histophysiology and immuno-intensity of androgen receptor in brown bear (*Ursus arctos*) in association with age, season, and spermatogenic score

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Abstract:
The brown bear (*Ursus arctos*) is a seasonal breeder with a mating season occurring in spring. However, there are reports on mating taking place also in Autumn. It has been shown that the timing of active spermatogenesis well coincides with the high reproductive activity. Some studies also indicated that the duration of active spermatogenesis was longer than that of the high serum testosterone level, and that the male reproductivity generally lasts longer than the female receptivity. The seasonal changes in spermatogenesis have been reported in American black bears and brown bears, and postulated that spermatogenesis occurs before, during, and after the mating season. Maintenance of spermatogenesis requires high testosterone. However, males are reported to produce spermatozoa actively despite low plasma testosterone concentrations and out of mating season. The testes of 38 free-ranging adult, young adult, and subadult male brown bears from human-caused mortalities in the Dinaric population were examined in spring and autumn. The aim of this study was to determine the factors that regulate testosterone production and contribute to differences in spermatogenetic activity, morphological, histological, and endocrinological changes. Testicular mass, volume, body mass, and testosterone concentrations in testicular tissue (using LCMS/MS technique) and hair samples (using EIA technique) were measured. The localization and immunohistochemical staining intensity of the 11β-hydroxysteroid dehydrogenase and androgen receptor (AR) were investigated using immunohistochemical methods. Seminiferous tubules diameter was significantly higher in the spring and was positively associated with the spermatogenic score ($p=0.001$). An increase in testicular mass and volume in adult males was observed in spring compared to adults in autumn, while no significant correlation between body mass, testes mass, and testicular volume was found. Our ongoing analyses will investigate the relation of spermatogenesis, testosterone biosynthesis, and other factors contributing to testicular activity in brown bear.

Understanding context and endocrine correlates of aggressive behavior in female mugger crocodiles during reproductive and non-reproductive phases

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Abstract:
Behavioral expressions vary across ecological contexts and in turn, influence physiological correlates in an organism. Here, we aimed to understand aggressive behaviors in female mugger crocodiles (*Crocodylus palustris*), the freshwater reptile largely distributed within the Indian subcontinent, and how such behaviors vary across different reproductive phases, from mounting to nesting to egg laying. We aimed to investigate the endocrine correlates, fecal testosterone and cortisol metabolites, for understanding the proximate mechanisms underlying such variations. Behaviors were recorded via CCTV camera from January to June 2022 for muggers housed in a zoo (Madras Crocodile Bank Trust, MCBT) in Chennai, India. We recorded behaviors (4320 hours of recordings across 180 days) from individuals belonging to a particular pen and as a part of the pilot study, we analyzed only dyadic (female-female) agonistic interactions in 12 hours of video recordings per day and two days/week for a month (N= 8). We also collected 103 scat samples from females and assays for testosterone and cortisol metabolites are ongoing. We defined two "event" behaviors, lunge and bite, and one "state" behavior, chase. Our results showed that aggression was significantly ($P<0.05$) different across the reproductive phases (from mounting to nesting to egg laying), with the highest total incidences (127) of lunge and bite and the longest duration (453 seconds) of chase during the phase of nest (hatchling/egg) defense. Variable agonistic interactions may represent altered physiological conditions and thus, may contribute towards understanding the plasticity of a trait, whether behavioral or physiological, within a given environment.

Glucocorticoids as potential biomarkers of stress in sei whale (*Balaenoptera borealis*) baleen

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Abstract:
Global warming can exacerbate the frequency and severity of climate events, leading to imbalances in the homeostasis of wildlife. Measuring deviations in circulating levels of glucocorticoids is a potential tool to investigate stressful episodes in mammals caused by either natural or anthropogenic sources. From February to April 2015, the largest baleen whale stranding ever recorded occurred along the coast of Chile, involving more than 300 sei whales. The whales were found to have ingested paralytic shellfish toxin, which resulted from a harmful algal bloom presumably triggered by a strong El Niño-Southern Oscillation (ENSO) event. This event is believed to have occurred over a period of approximately three to six months. The aim of the present study was to retrospectively examine whether cortisol and corticosterone levels exhibited any detectable increase in the baleen plates of four individual sei whales prior to their death. The lengths of baleen plates ranged from 48 to 52 cm. Plates were sub-sampled at 4 cm intervals, a baleen growth period encompassing the final three years of each individual whale's life. Preliminary results indicate that corticosterone levels remained consistently low over the years (≤ 1.61 ng/g) but were elevated in the last six months of life, with mean values over 2.96 ng/g in all whales. Cortisol elevations were observed in two of the four whales in the last two months of life. Further investigations are currently underway to determine whether cortisol or corticosterone is the dominant glucocorticoid associated with the endocrine stress response in this species.

Human activity and wildlife tourism: significant contributors to elevated glucocorticoid levels in *Panthera tigris tigris* (Tiger) in India

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Abstract:
India has 74,715 km^2 of total Protected Area for Tigers, out of which 41,500 km^2 belongs to the core tiger habitat and the remaining 33,222 km^2 belongs to the buffer region. In both these regions, Human habitation and activity including wild-life tourism are present, buffer having more such occurrences than in core area. To understand the impact of these anthropogenic disturbances and wild-life tourism on stress physiology of tigers, from five big tiger reserves more than 700 tiger scat samples were collected from the forest floor of these protected areas to measure the glucocorticoid metabolite concentrations, non-invasively, for over a period of 2 years during tourism and non-tourism seasons. The steroid hormone metabolites were extracted and measured using standardized Enzyme Linked Immuno Sorbent Assay. The data corresponding to the human activities and tourism were recorded and also collected from the forest department of each park. In most of the above chosen parks, core area has significantly higher stress compared to that in buffer area ($p<0.05$), during tourism season. In the core area, stress levels are significantly higher during tourism period compared to non-tourism period ($p<0.05$). In Tadoba-Andheri Tiger Reserve, buffer region showed significantly higher stress levels during non-tourism period when compared to tourism period ($p<0.05$), whereas in Jim Corbett Tiger Reserve, the buffer region has significantly higher stress levels during tourism season when compared to non-tourism period($p<0.05$). Most of the stress hotspots are located near the road junctions, tourism path and trails and near the core and the buffer boundaries. In conclusion, long-term elevation of fecal glucocorticoid levels corresponding to the presence of disturbances and tourism suggests that human activity and wild-life tourism are major factors in causing stress to Tigers. Managing tourism and controlling human activities can alleviate the stress levels in Tigers and lead to the overall well-being.

Stress in the Anthropocene: the complex relationship between physiological stress and anthropogenic food consumption in sika deer

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Abstract:
Consumption of anthropogenic foods, such as crops and pasture grass, by wildlife has been observed around the world. Reliance on anthropogenic foods enhances physical and reproductive performance of wildlife. In contrast, to access these foods, they need to move into human-modified environments. This, in turn, can impose physiological stress on them. However, it remains largely unknown how consumption of anthropogenic foods affects the physiological conditions. In this study, we investigated the relationship between stress levels and anthropogenic food consumption in sika deer *Cervus nippon*. Hair samples were collected from deer captured through nuisance control and hunting in Nagano prefecture, Japan, between January and April 2020. We performed hair cortisol analysis to evaluate stress levels and nitrogen stable isotope analysis to investigate the relative dietary contribution of anthropogenic foods. We tested the effects of nitrogen stable isotope ratios ($\delta^{15}N$), sex, and body condition index (BCI) on hair cortisol concentrations (HCC) for each age class (juvenile or adult). In adult females, we also investigated the effect of HCC on reproductive conditions, i.e., pregnancy rate. We found the significant negative effect of BCI on HCC, but no effects of sex and $\delta^{15}N$ value in juvenile deer. Furthermore, we found the significant sexual differences and positive effects of hair $\delta^{15}N$ value on HCC in adult deer. However, as the interaction between sex and $\delta^{15}N$ value significantly affected HCC, we reanalyzed the relationship between HCC and $\delta^{15}N$ value for each sex. Our reanalysis revealed that female deer with high $\delta^{15}N$ value showed high HCC, but males did not. We also found no effect of HCC on reproductive conditions. These findings suggest that the use of anthropogenic foods can cause physiological stress depending on life history stages and sex. However, it seems that such stress does not necessarily reduce fitness.

Development of an ISWE 11-oxoetiocholanolone mini-kit for the non-invasive quantification of glucocorticoid metabolites in feces

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Abstract:
As part of the ISWE mission to promote the field of wildlife endocrinology, a goal of our society is to develop antibodies and enzyme immunoassay kits for the community that are cost-effective and facilitate research in a diverse range of species and sample matrices. One particular need highlighted by our membership is for a greater variety of assays for the quantification of glucocorticoid metabolites, especially incorporating group-specific antibodies that can have increased sensitivity for assessing an adrenal response to potential stressors. An antibody against 11-oxoetiocholanolone-17-CMO was first generated in rabbits, and along with our partners at Arbor Assays, Inc., an ISWE mini-kit incorporating a HRP-conjugated label and 11-oxoetiocholanolone standard has been developed, designed for use with anti-rabbit IgG secondary antibody coated plates. This mini-kit is suitable for quantifying glucocorticoid metabolites with a 5β-3α-ol-11-one structure and has been tested with fecal extracts from 25 species, including African and Asian elephants, American bison, Ankole cow, blue wildebeest, blue and yellow macaw, brushtail possum, cape buffalo, chamois, fat-tailed dunnart, Florida manatee, ghost bat, giraffe, golden langur, gorilla, Gould's wattled bat, hippopotamus, Krefft's glider, Leadbeater's possum, mandrill, okapi, roan antelope, samango monkey, short-beaked echidna and tiger. Responses to biological and pharmacological challenges are compared with data obtained via alternative glucocorticoid immunoassays. Here we present the outcome of this beta-testing, and recommendations for using this mini-kit in further species and sample types by the ISWE and wider community.

Quantifying corticosterone in bird feathers to assess chronic stress levels in avian populations inhabiting eastern Himalayan forests

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Abstract:
Chronic stress is known to significantly impact wildlife populations, potentially affecting their health and survival. We aimed to assess the chronic stress levels in bird populations residing in two distinct forest types: primary forests and logged forests at Eaglenest Wildlife Sanctuary in the Eastern Himalayas. Relative to primary forests, logged forests have elevated temperatures throughout the year which might contribute to chronic stress in birds. We have collected feather samples from multiple bird species living in primary and logged forests. We have isolated corticosterone, a key marker of stress from bird feathers. Corticosterone circulated in the blood is sequestered in the feathers, and therefore corticosterone extracted from bird feathers serve as a key marker of chronic stress. Subsequently, the isolated corticosterone will be quantified to allow us to compare the chronic stress experienced by the birds in the two forest types. Further, we have also measured growth bar lengths on the bird feathers to help us understand the feather growth rates. Growth bars are elusive, alternating dark and light bands most seen in wing and tail feathers. A pair of growth bars correspond to 24 hours of feather growth. By measuring the width of growth bars, we estimate the time required for the feather to grow. This study's findings may have significant implications for avian conservation and management strategies. By establishing a potential link between chronic stress and forest degradation, our research contributes valuable insights into the health of bird communities. Further, the study also aims to link chronic stress in birds to feather growth rates. Ultimately, this knowledge can guide conservation efforts aimed at preserving avian biodiversity and ecosystem health in the face of increasing anthropogenic disturbances.

Behavioral and endocrine correlates in a reintroduced population of swift foxes (*Vulpes velox*)

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Abstract:
The swift fox (*Vulpes velox*) was extirpated from the northern Great Plains of North America in the early 1900s due to agricultural expansion and persecution. A series of reintroductions have restored the species to parts of its range, though it remains regionally threatened. A reintroduction is currently underway at the Fort Belknap Indian Reservation, Montana; however, minimal data exists on the effect of behavioral and endocrine traits on the health and survival of translocated foxes. This study explores relationships among stress and nutritional physiology, bold personality, post-release movement, and survival in the Fort Belknap population. Behavioral observations and fecal samples (n = 317) were collected from 76 wild-sourced foxes that were translocated between August and September of 2021 and 2022. Samples were lyophilized and fecal glucocorticoid metabolites (fGMs) extracted by shaking in 90% ethanol. fGM concentrations were then quantified using a cortisol enzyme immunoassay (C. Munro, R4866). All procedures were approved by the Smithsonian Institution Animal Care and Use Committee (NZP-IACUC 20-09). Preliminary data analyses included linear regression, two-sample pooled variance t-tests, and ANOVAs. Results from the 2021 release cohort suggest fGM concentrations may differ between source populations, age, and sex, but not with boldness. Additionally, fGMs may be associated with post-release survival, but not movement. To further elucidate physiological responses to translocation, subsequent analyses will incorporate data from the Wyoming source population in 2021, the 2022 release cohort, as well as fecal triiodothyronine metabolite (fT3) concentrations quantified from an Arbor Assays T3 enzyme immunoassay kit (K056-H1). This study demonstrates the use of behavioral and endocrine correlates as a tool for *in situ* conservation efforts.

Habitat-dependent foraging behaviour of Nilgiri langurs (*Semnopithecus johni*) and their stress status in the Nilgiris: a comparative preliminary study

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Abstract:
Nilgiri langurs (NLs; *Semnopithecus johnii*) are "vulnerable" (IUCN Red Data book) primates, endemic to evergreen sholas of the Nilgiris (altitude: 1200 - 2600 m). Being highly arboreal, NLs feed on leaves, flowers, and nuts of a variety of endemic plants in shola forests of Thai Shola and Carrington/Kinnakorai Shola. However, their numbers have been reducing because of habitat destruction and other anthropogenic events. Besides, NLs, living in fringe areas of shola forests proximal to human-dominated landscapes and farmlands such as Mullikorai and Nanjanad areas have adopted to opportunistic feeding on 'cash-crops' (carrots, cabbage and potato; tubers). Consequently, the latter ones have been under conflict with farmers and experience varied stresses due to chasing and intimidation by farmers and pet-canines. This has contributed to apparent-behavioural/physiological alterations in NLs which have not been assessed. We conducted a ten-month preliminary observational study in the above two landscapes *viz*., undisturbed protected area (U-PA) and human-inhabited area (H-IA). Study parameters included sighting of NLs, their troop size-composition, foraging behaviour, and collection of fecal samples for analysis of glucocorticoid metabolites (GCMs). Our results show that number of NL troops sighted was significantly more in U-PA *vis-à-vis* H-IA (25 *vs* 7); but numbers of individuals per troop were similar (range: 6-23) in both areas. In the U-PA, animals foraged on Acacia mearnsii, Daphniphyllum neilgherrense, Eucalyptus globulus and others. Moreover, visual-photographic evidence indicated that U-PA's NLs were larger and robustly built than those in H-IA. To assess the stress status of NLs, fecal samples (n = 48) were collected and GCMs are to be measured. Our preliminary observational studies indicate that there are habitat-dependent changes in the physical build up, foraging behaviour and stress status of NLs inhabiting forests of U-PA and H-IA. (Tamil Nadu Govt. Permission no. 2022/40, dated 23rd June 2022).

A change of heart: seasonal trends in heart rate and hair thyroid hormones in maned wolves (*Chrysocyon brachyurus*)

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Abstract:
As wildlife species face unprecedented rates of environmental change, we must rely on integrated physiological metrics to assess their health and well-being. Maned wolves (*Chrysocyon brachyurus*) experience broad-scale landscape alteration, presenting a need for reliable indices of metabolic health. Following successful deployment of Reveal LINQ™ (Medtronic Inc., Minneapolis, MN) biologgers in maned wolves *ex situ*, we describe seasonal trends in heart rate (HR) and hair triiodothyronine (T3). Hair samples were collected approximately every three months for one year from maned wolves (n=8) implanted with biologgers at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, Virginia, USA. Biologgers recorded two-minute HR averages, which were averaged across periods between hair collection dates. T3 was measured in guard hair via a T3 enzyme-linked immunosorbent assay (Arbor Assays, MI), which passed parallelism validations for maned wolf hair extract. We evaluated the effect of season and sex on HR and T3 separately using linear mixed models. Though seasonal patterns in HR differed slightly by sex, both males and females displayed elevated HR in winter. Only females displayed a similar pattern of increased T3 in winter. These findings may indicate an increase in female maned wolves' metabolic rate during this period, potentially related to temperature regulation or reproductive activity, among other factors. Ultimately, we demonstrate the validity of our methods to extract and measure T3 in maned wolf hair, but also a need for larger sample sizes to detect seasonal effects. Because hormones are incorporated into hair as it grows, it is a particularly valuable sample type for retrospective analysis of animals' physiological state over time. Thus, this integrated assessment of heart rhythms and endocrinology shows promise for application *in situ*, in combination with other ecological assessments, as we aim to understand how free-ranging maned wolves meet the energetic demands of altered landscapes.

Studying hormones in one of the most elusive, deepest-diving species on the planet, Blainville's beaked whales (Mesoplodon densirostris)

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Abstract:
Beaked whales (family *Ziphiidae*) are mysterious marine mammals – they are deep-diving species, rarely seen at sea and can spend long periods underwater hunting squid and fish at depths of 2,000 m below the surface. Because of their auditory biology and diving behavior, beaked whales are known to be sensitive to naval sonar exposure. This sensitivity raises serious concerns for the conservation of beaked whales inhabiting U.S. Navy testing ranges or regularly exposed to sonar exercises. The impact of disturbance on cetacean populations has predominantly been quantified through strandings and mortalities, or changes in distribution. Hormone data from these animals could afford managers significant insight into the biological processes of conservation concern, such as stress responses and reproduction. A major challenge in endocrine studies of cetaceans (especially beaked whales) is that sample types conventionally used for hormone analysis (i.e., blood) are not feasible to collect from free-swimming individuals. Given the challenges of studying an elusive, deep-diving species and the importance of obtaining data, there is a need to advance techniques that will address significant gaps in our knowledge on beaked whales. Here, we present on our work to develop a method for collecting and analyzing hormones in exhaled breath (blow) from beaked whales during surfacing intervals. Using a 7-m handheld pole, we have successfully collected 21 blow samples from individual Blainville's beaked whales, off Abaco Island, The Bahamas – a well-known population with documented life histories and some hormone profile information (i.e., fecal and blubber hormones). We will use enzyme immunoassay to investigate three steroid hormones (testosterone, progesterone, and cortisol) in blow samples, as key indicators of adrenal activation and reproductive activity. Our findings are a fundamental step towards developing methods to assess the physiology of vulnerable beaked whale populations.