

Development and Application of Physiological Markers of Grizzly Bear Health



Abbey Wilson¹, David Janz¹, Luciene Kapronczai², Gordon Stenhouse³

¹Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5B3

²Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada S7N 5B3

³FRI Research Grizzly Bear Program, Hinton, Alberta, T7V 1V3



UNIVERSITY OF SASKATCHEWAN
Western College of
Veterinary Medicine
DEPARTMENT OF VETERINARY BIOMEDICAL SCIENCES
USASK.CA/WCVM



fRI Research
Informing Land & Resource Management



Bachelor of Science, Biology (minors in Chemistry and Spanish)

August 2008 – May 2012 (January 2011 – May 2011)

Sweet Briar College, Sweet Briar, VA, USA (University of Seville, Spain)

*Pollinator visitation and richness between male and female *Thalictrum pubescens**

Large Animal Veterinarian Assistant

January 2012 – April 2013

Virginia Equine Services, Lynchburg, VA, USA

Memphis Zoo Research and Conservation Intern

May 2013 – October 2013

Memphis Zoological Society, Memphis, TN, USA

Assessment of reproductive status by steroid monitoring and mating behavior

Doctor of Philosophy, Animal Physiology Interdisciplinary Program

January 2014 – December 2017

Mississippi State University, Mississippi State, MS, USA

Memphis Zoological Society, Memphis, TN, USA

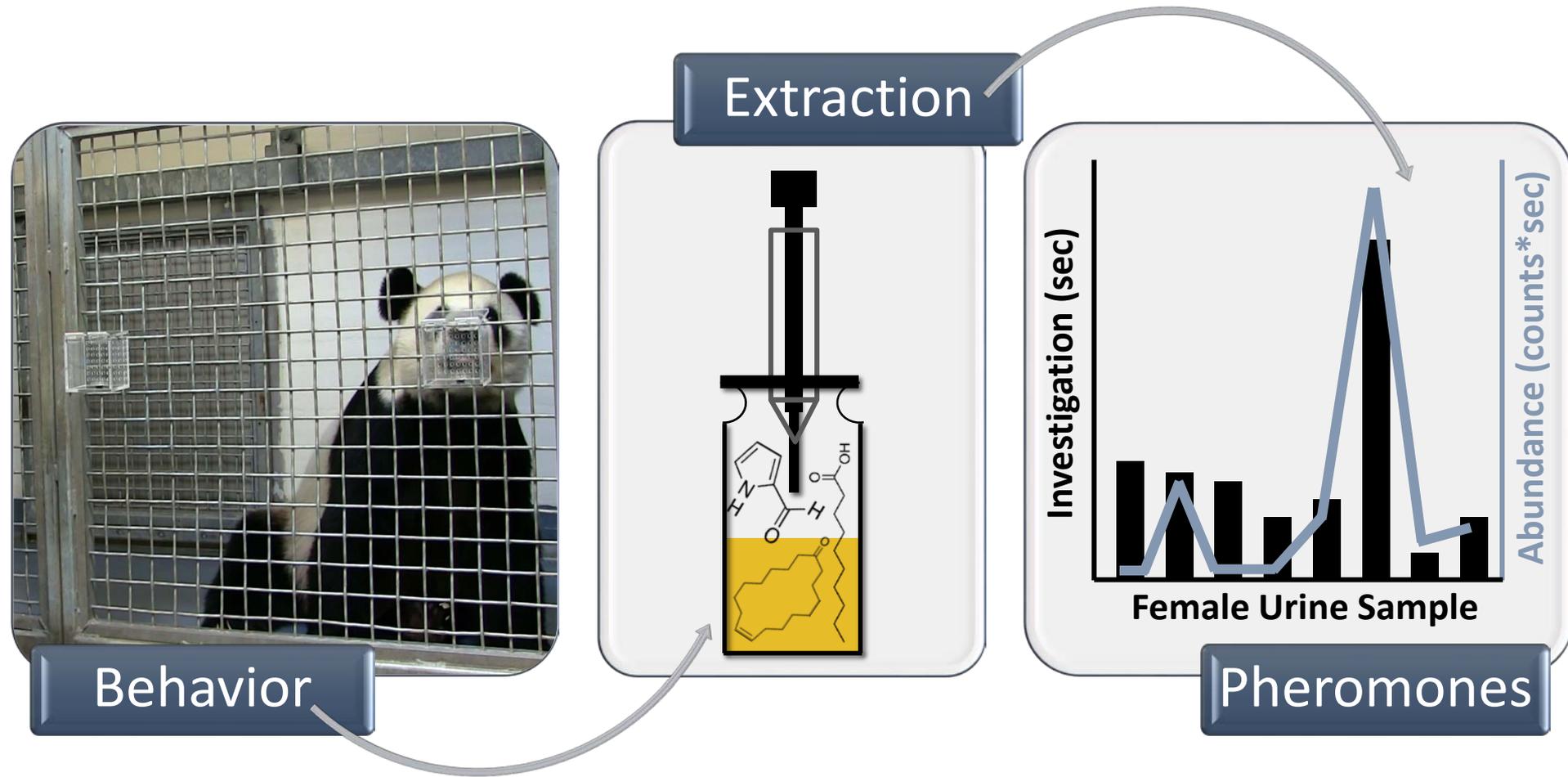
Chemical signaling by giant pandas to communicate sexual receptivity

Postdoctoral Associate

January 2018 – June 2018

Mississippi State University, Mississippi State, MS, USA





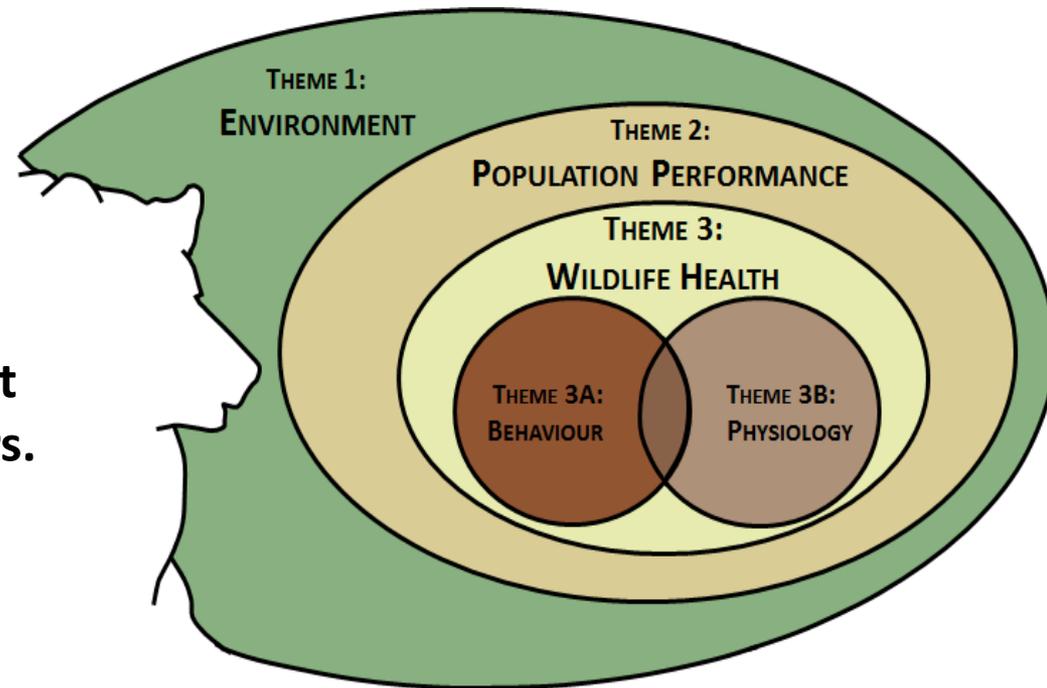


01/04/2011 12:42:44

4. Have changing landscape conditions associated with anthropogenic natural resource extraction activities resulted in changes in habitat selection by, and the health of, grizzly bears within the study area?

Q3A.1, Q3B.1, Q3B.2

We want to determine if resource extraction activities and natural disturbances in the landscape affect the health of individual grizzly bears.





1. On different landscapes
2. Over time on different landscapes
3. Relative to other measures of physiological function

Objective 1: Develop a liquid chromatography/tandem mass spectrometry/multiple reaction monitoring (LC/MSMS/MRM) method to identify and quantify the expression of 12-16 proteins in skin that are associated with reproduction, energetics, and stress.

Objective 2: Determine concentrations of hormones in hair that are associated with reproductive function (testosterone, progesterone, estradiol) and long-term stress (cortisol).

- Conservation medicine: the application of 21st century technologies used in human medicine (for disease diagnosis, biomarker discovery, and drug development) to free-ranging wildlife species.
- Population dynamics (birth rate, mortality rate, immigration, and emigration) are controlled by the fitness of individual animals within populations.

RESEARCH ARTICLE

Non-invasive identification of protein biomarkers for early pregnancy diagnosis in the cheetah (*Acinonyx jubatus*)

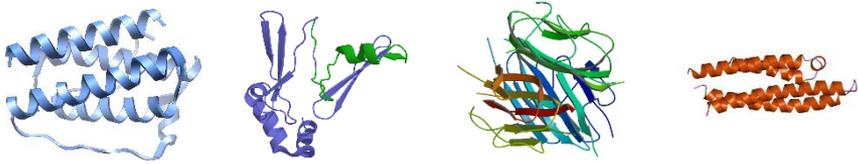
Diana C. Koester¹▫, David E. Wildt¹, Morgan Maly¹, Pierre Comizzoli², Adrienne E. Crosier¹*

¹ Center for Species Survival, Smithsonian Conservation Biology Institute, Front Royal, VA, United States of America, ² Center for Species Survival, National Zoological Park, Washington, DC, United States of America

▫ Current address: Conservation and Science Department, Cleveland Metroparks Zoo, Cleveland, OH, United States of America

* CrosierA@si.edu

- Identified protein profile present in fecal material of females.
- Isolated proteins that may be candidates for early pregnancy diagnosis.



Leptin

Ghrelin

Adiponectin

Apolipoprotein

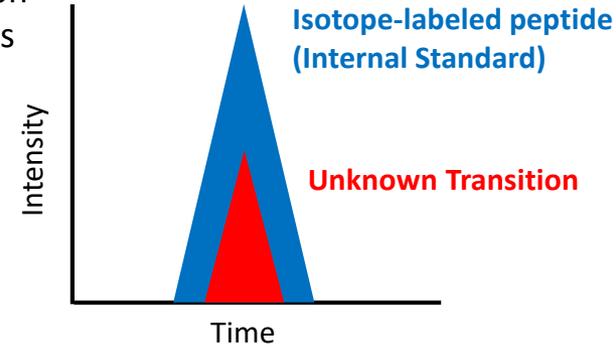


1. Identify target proteins (corresponding unique peptides) as candidate biomarkers in *Ursus arctos*

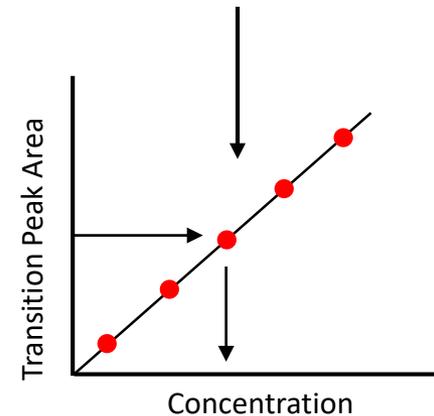
2. Complete trypsin digestion to separate distinct peptides

4. Create a standard curve using an internal standard

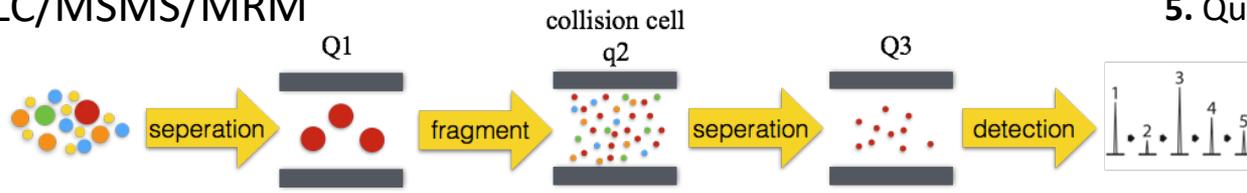
3. Develop MRM assay with University of Victoria Genome British Columbia Proteomics Centre using a Thermo Scientific EASY-nLC II coupled with an LTQ Orbitrap Velos-Pro mass spectrometer



5. Quantify concentration of unique peptides



LC/MSMS/MRM

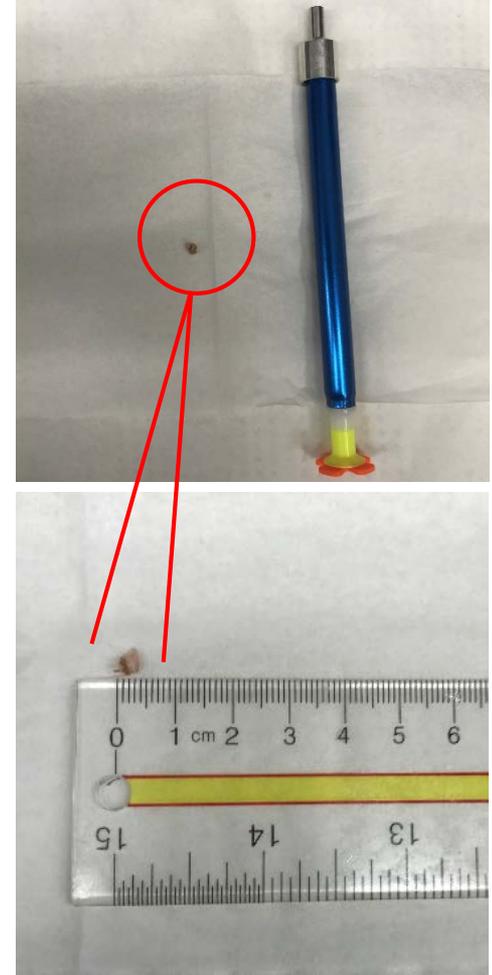


Precursor ion m/z 400.24
(Distinct peptide from Trypsin/LC: Electron ion spray)

Product ion m/z 205.21
(Corresponding fragment ions from collision energy in mass analyzer)

Transition: 400.24 / 205.21
Amount of product ion = Amount of corresponding protein

- Since 2013, 139 skin samples from 112 individuals have been collected by ear plug and/or skin biopsy from free-ranging grizzly bears in our study area.
- Developed extraction technique to isolate and purify sufficient protein from small (<0.1g) skin biopsies.
- Initial analysis completed by University of Victoria Genome British Columbia Proteomics Centre revealed 612 proteins present in skin, 546 of which are also found in other bear species, and 8 of those are in our target list.



Next Steps: Complete additional discovery runs to refine target protein list, develop LC/MSMS/MRM assay, and relate changes to other theme data in the project (environmental, nutritional, and behavioral variables).



Grizzly Bear Proteomic Profile

Bear ID: G170

Collection Date: 08May2017

Test	Result	Flag	Normal Range (park bears)	Description (H: high/L: low)
Energetics				
Leptin	15.6		9.0-29.0	
Ghrelin	0.9		0.4-1.4	
Adiponectin*	4.2	L	9.0-12.2	
Apolipoprotein*	3.0		1.9-5.0	
Reproduction				
Follicle Stimulating Hormone	50		0-120	
Luteinizing Hormone	23		0-60	
Relaxin	42		0-140	
Ceruloplasmin*	15	H	0-13	
Stress				
Heat shock protein 70*	159	H	141-152	
Heat shock protein 90*	4.0		3.8-5.3	
α2macroglobulin*	110		102-120	
Corticosteroid-binding globulin*	115		75-125	
Kininogen*	186		120-310	

- Steroid hormones accumulate in the hair shaft during hair growth and represent a long-term “chronology” of circulating hormone over months.
- Relate cortisol concentrations to reproduction, immune function, and growth and sex steroid hormones to sex, age class, and reproductive status.



Contents lists available at [ScienceDirect](#)

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

- Hair cortisol concentrations predicted pregnancy and longer term cortisol may influence reproduction.
- Reducing cortisol prior to commencing IVF may improve outcomes.

Relationship between hair and salivary cortisol and pregnancy in women undergoing IVF

Adam J. Massey^a, Bruce K. Campbell^b, Nick Raine-Fenning^{b,c}, Catherine Pincott-Allen^b, Jane Perry^a, Kavita Vedhara^{a,*}

^a Division of Primary Care, School of Medicine, University of Nottingham, UK

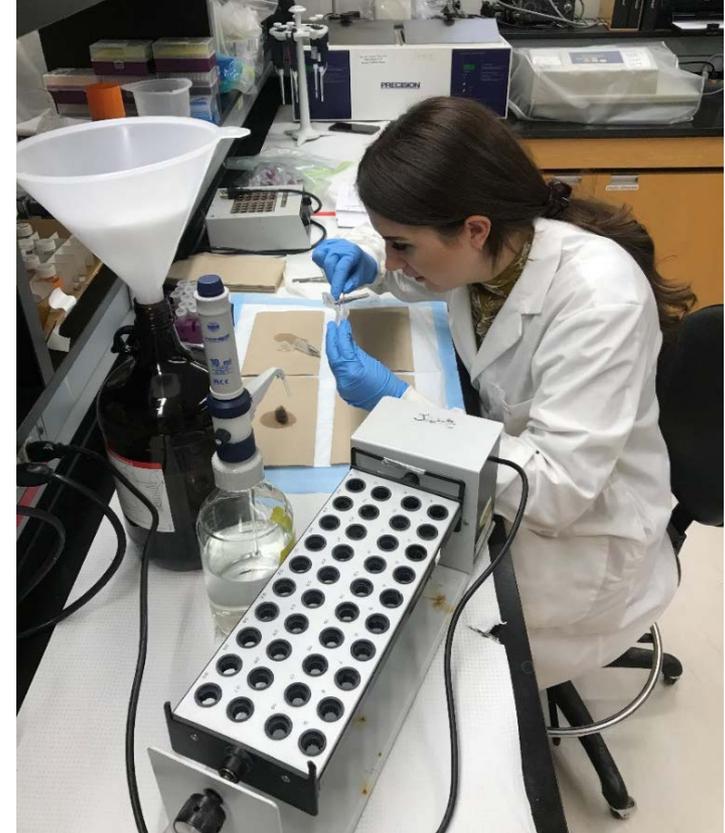
^b Division of Child Health, Obstetrics and Gynaecology, School of Medicine, University of Nottingham, UK

^c Nurture Fertility, UK



- Guard hairs are collected by shaving or cutting with scissors a minimum of 5cm x 5cm (2in x 2in) patch of hair from the left side of the hump (area between the shoulder blades) and/or by barbed-wire hair snags.
- Hair is ground into a fine powder and hormones are extracted with methanol.
- Hair hormone concentrations are measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits and a SpectraMax plate reader.

- Since 2000, 374 hair samples from 288 individuals have been collected by shaving and/or barbed-wire snag from free-ranging grizzly bears in our study area.
- Extraction techniques have been developed and validated in our laboratory to isolate hormones in hair.
- All hormone concentrations have been determined for samples collected through September 2017.



Next Steps: Application of the hair hormone analyses will be integrated with other theme data in the project (environmental, nutritional, and behavioral variables).

Grizzly Bear Hormone Diagnostic Screen

Bear ID: G313

Collection Date: 14Jun2017

Test	Value	Flag	Baseline Range (pg/mg)*	Conclusion
Reproduction				
Testosterone	23.40	High	0.483 - 20.840	Adult Male
Progesterone	18.41		4.570 - 18.409	
Estradiol	0.026		0.006 - 0.026	

Stress

Cortisol	50.44	High	0.160 - 18.187	Increased Stress
----------	-------	------	----------------	------------------

Landscape variables under investigation

Oil/gas (well sites)	<ol style="list-style-type: none"> 1. Provide information on sex, age class, and reproductive status. 2. Determine if individual shows physiological stress. 3. Relate changes to landscape disturbances. <p>*Baselines will vary by sex, age class, and season</p>
Forestry	
Coal	
Pipelines	
Road density	
Recreation	

**Baseline range: minimum and maximum values of hormone concentrations within (+/-) 2 standard deviations of the mean from data analyzed up to Sept 2017 collections.*

4. Have changing landscape conditions associated with anthropogenic natural resource extraction activities resulted in changes in habitat selection by, and the health of, grizzly bears within the study area?

Q3A.1, Q3B.1, Q3B.2

- Goal: Create a novel tool to monitor species-at-risk on changing landscapes that can be communicated efficiently with industry partners.
 1. Create proteomic profiles for individual grizzly bears that provide information on energetics, reproduction, and stress of the animal.
 2. Relate changes in hair hormone concentrations to landscape disturbance and other themes of the project.
 3. Link changes in skin and hair health indicators to (1) landscape change, (2) landscape change over time, and (3) other measures of physiological function to create an overall health assessment for each individual, which will inform management decisions.



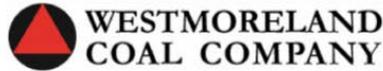
Teck



SEVEN GENERATIONS
ENERGY LTD



West Fraser



WESTMORELAND
COAL COMPANY



FOREST RESOURCE
IMPROVEMENT
ASSOCIATION OF ALBERTA



Alberta
Newsprint
Company



Weyerhaeuser



UNIVERSITY OF SASKATCHEWAN
Western College of
Veterinary Medicine



NSERC
CRSNG



fRI Research
Informing Land & Resource Management

DEPARTMENT OF VETERINARY BIOMEDICAL SCIENCES
USASK.CA/WCVM



Abbey E. Wilson, Ph.D.

Postdoctoral fellow

Department of Veterinary Biomedical Sciences

University of Saskatchewan, Saskatoon, SK, Canada S7N 5B3

E: abbey.wilson@usask.ca; P: 306-966-7762

Questions?

Thank you!