2019 Rhino Research Council

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TAG Chair: Adam Eyres, Fossil Rim Wildlife Center, TX

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Co-Advisor: **Paul Reinhart**, Cincinnati Zoo, OH (Sumatran rhino)
Co-Advisor: **Randy Rieches**, San Diego Zoo Global, CA (White rhino, GOH)
Co-Advisor: **Lisa Smith**, Buffalo Zoo, NY (Black rhino)
Co-Advisor: **Clarice Brewer**, White Oak Conservation Center, FL

Genetics

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Co-Advisor: **Dr. Alfred Roca**, University of Illinois, IL
Co-Advisor: **Dr. James Austin**, University of Florida, FL
Co-Advisor: **Dr. Candace Scott**, Queens University, Canada

Health (Veterinary Medicine)

Advisor: **Dr. Michele Miller,** Stellenbosch University, South Africa
Advisor: **Dr. Eric Miller,** St. Louis Zoo, MO
Co-Advisor: **Dr. Benn Bryant**, Taronga Conservation Society, Australia
Co-Advisor: **Dr. Robin Radcliffe**, Cornell University, NY
Co-Advisor: **Dr. Beth Hammond**, Lion Country Safari, FL

Nutrition

Advisor: **Dr. Katie Sullivan**, Disney's Animals, Science and Environment, FL
Co-Advisor **Dr. Marcus Clauss**, University of Zurich, Switzerland
Co-Advisor **Dr. Ellen Dierenfeld**, Ellen S. Dierenfeld LLC, MO
Co-Advisor **Kerrin Grant**, The Wildlife Center, NM
Co-Advisor **Barbara Henry**, Cincinnati Zoo, OH
Co-Advisor **Dr. Eduardo V. Valdes**, Disney's Animals, Science and Environment, FL

2019 Rhino Research Masterplan

Primary Research Priorities

I. Impact of and control over body condition/weight

- Reproductive dysfunction
- Foot/joint problems
- Phytoestrogen relationship
- Diet composition and variety versus nutrient composition impact
- Health/disease impacts
- Overall effect on well-being
- How to best monitor/measure and alter to improve animal well-being

II. Iron overload in browsing rhinos

- Epidemiological review – what really is the significance/prevalence of IOD?
- Best biomarkers for detecting, monitoring and assessing condition or treatment
- Organ iron accumulation versus organ damage
- Association with other health issues (is it primary or secondary?)
- Interaction with other micro-nutrients

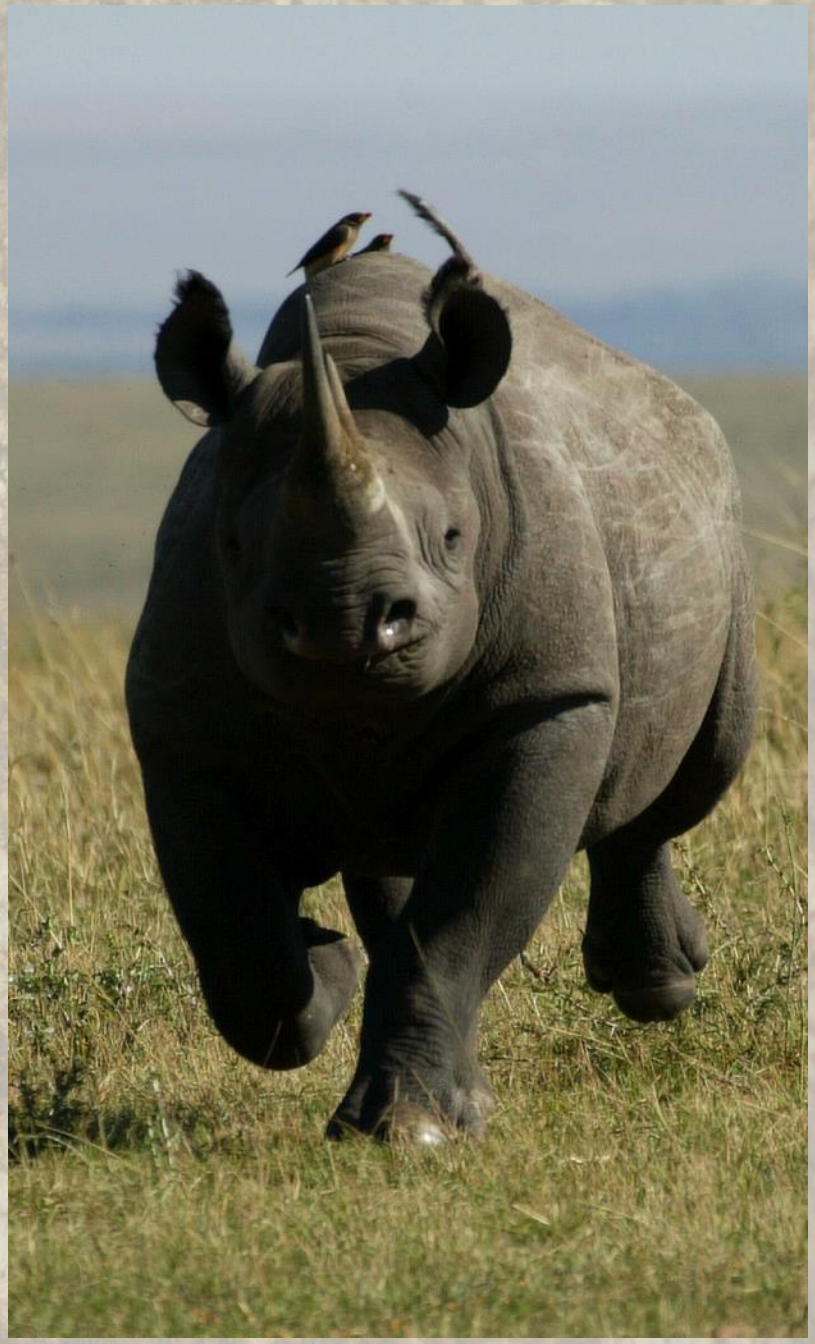
Primary Research Priorities Cont.

III. Understand/address early and late stage reproductive dysfunction

- Impact of over-conditioning
- Cause of stillbirths/pregnancy loss
- Why so much cyclic dysfunction (silent estrus, acyclicity, anovulation)?

IV. Investigate behavioral and environmental factors that affect rhino well-being

- *Ex situ* health, body condition, reproduction, socialization, enriched environment
- *In situ* impact of factors like dehorning and traumatic injury recovery



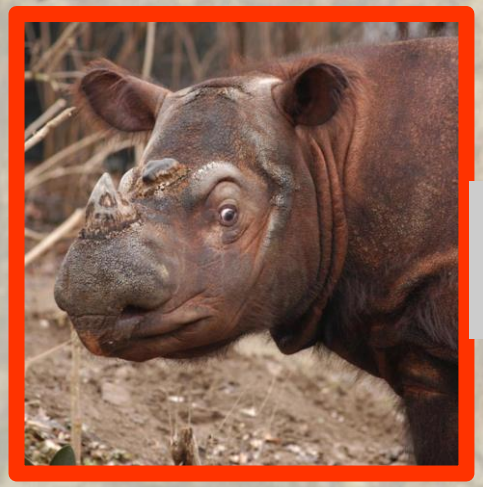
RHINOCEROS RESEARCH MASTERPLAN
2019

Association of Zoos and Aquariums
Rhinoceros Taxon Advisory Group
Rhino Research Council

Rhino Research Masterplan sent to all Rhino IRs

Contact Terri, Adam or any RRC Advisors for document

Iron overload disorder in rhinos – study updates



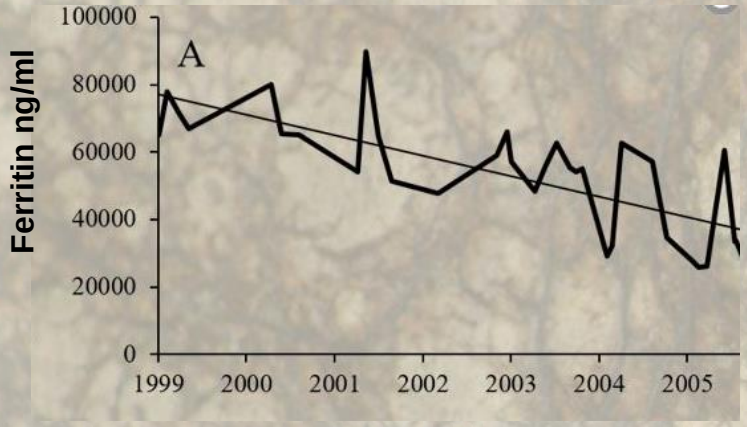
Sumatran

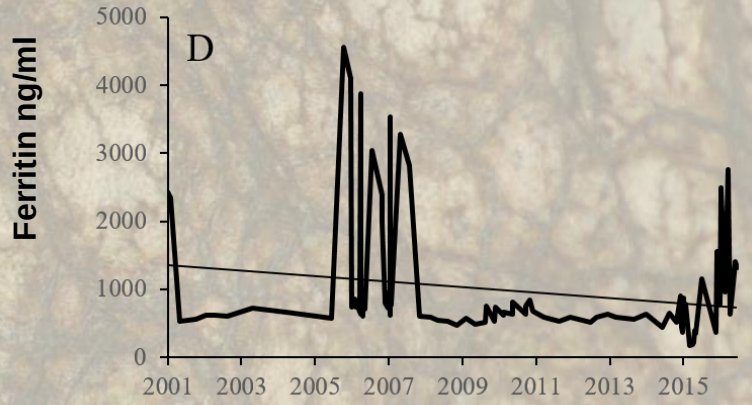
White

Black

Greater-one horned GOH

WHAT NEXT?



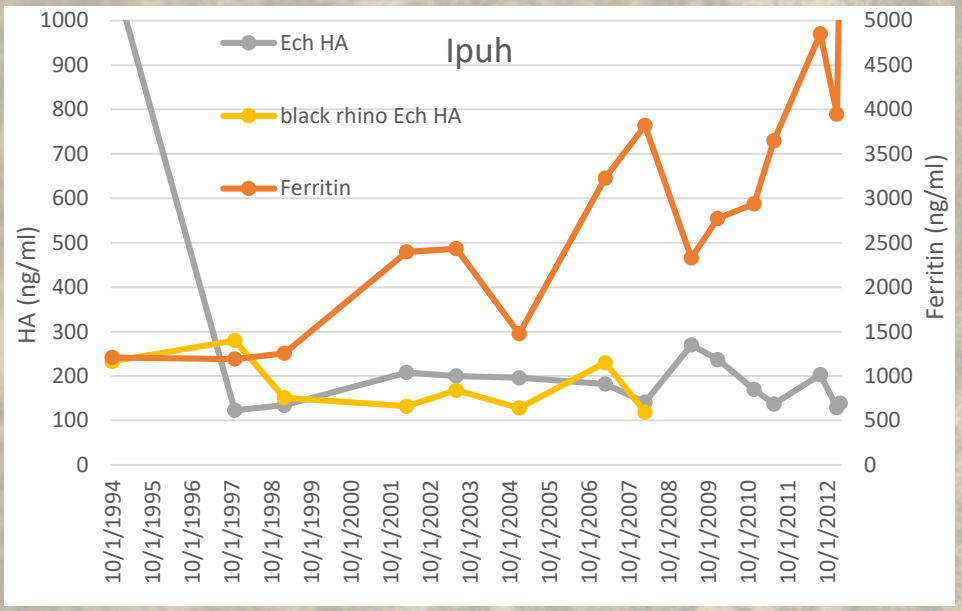


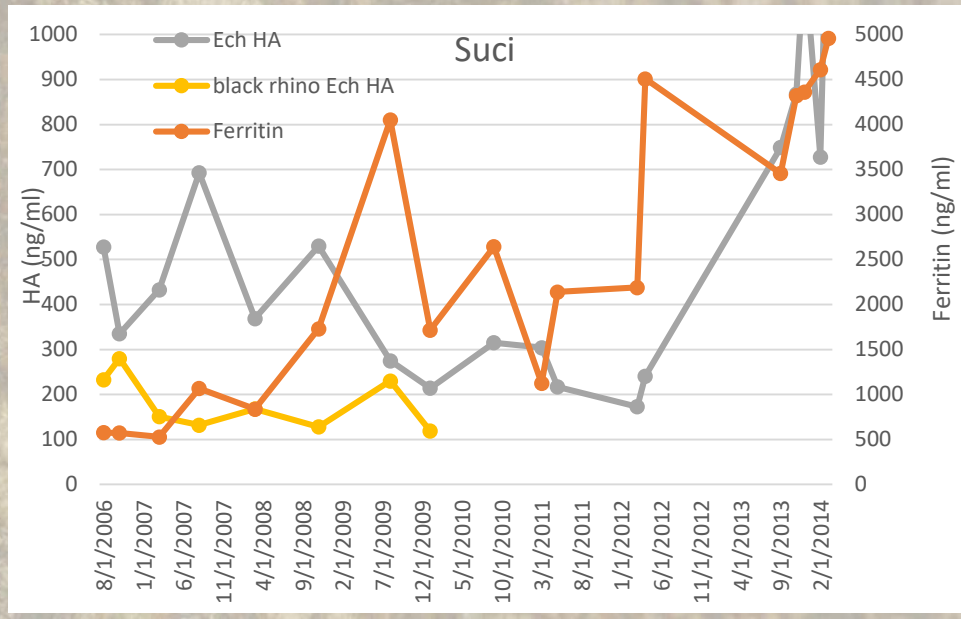
Previous studies in Sumatran and black rhinos indicated serum ferritin is not a good biomarker of IOD progression and severity.

Other Tests (preliminary data)

Hyaluronic Acid - filtered by the liver

Best biomarker to-date but may be too late; probably indicates general liver crisis; not specific to IOD





Labile Plasma Iron (LPI)

- Non-protein bound iron
- Induces Fenton reaction = reactive oxygen species (ROS)
- Causes cell and tissue damage
- LPI should never be positive in healthy individuals

Results:

Sumatran rhinos

- 15 samples from 3 Sumatran rhinos
- 3 samples were LPI positive
- All 3 were from 2 rhinos clinically sick with IOD

Black rhinos

- 16 samples from 6 black rhinos
- Results variable/inconsistent
- 1 individual sick with IOD was positive for LPI
- 2 individuals considered healthy were positive for LPI
- 1 healthy rhino had very high LPI one month and was negative for LPI the next

A side observation that may be important

Iron chelator — Iron chelator

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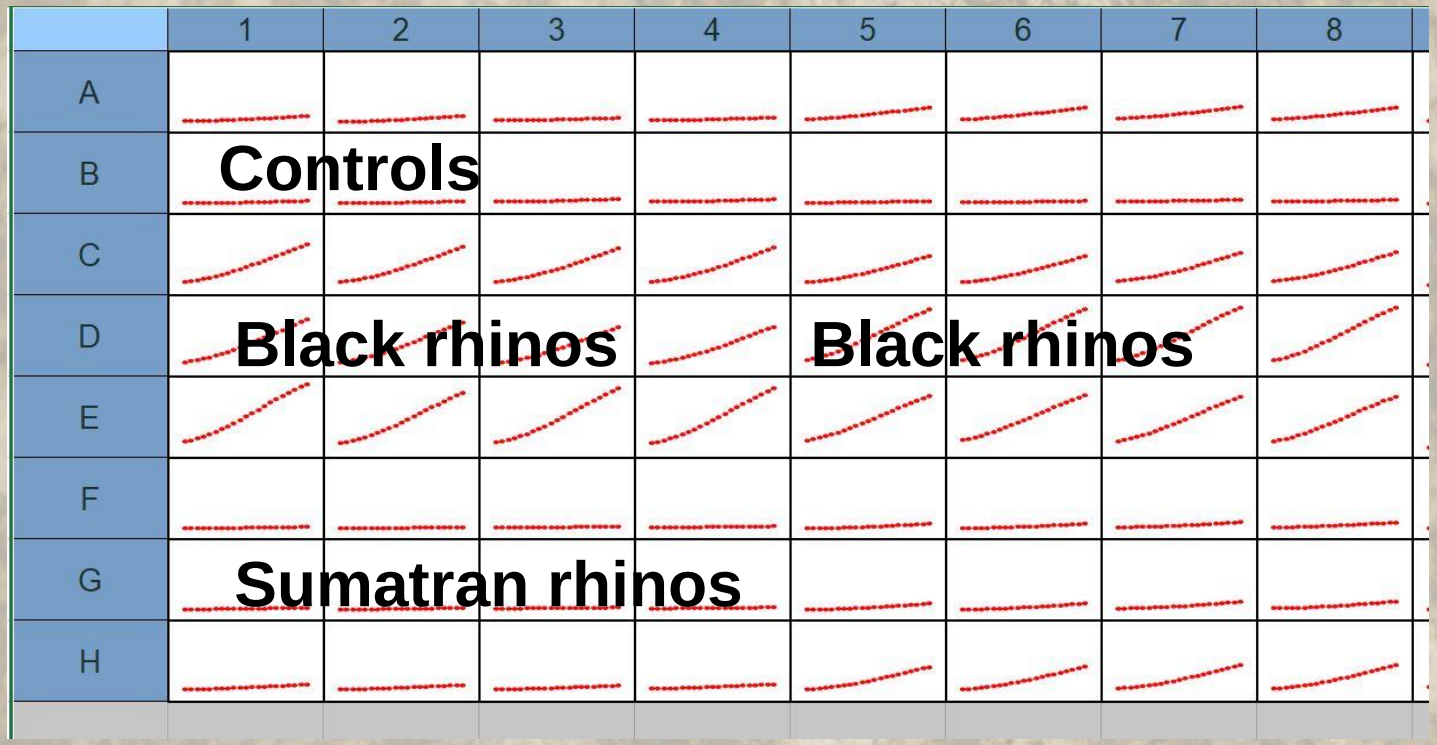
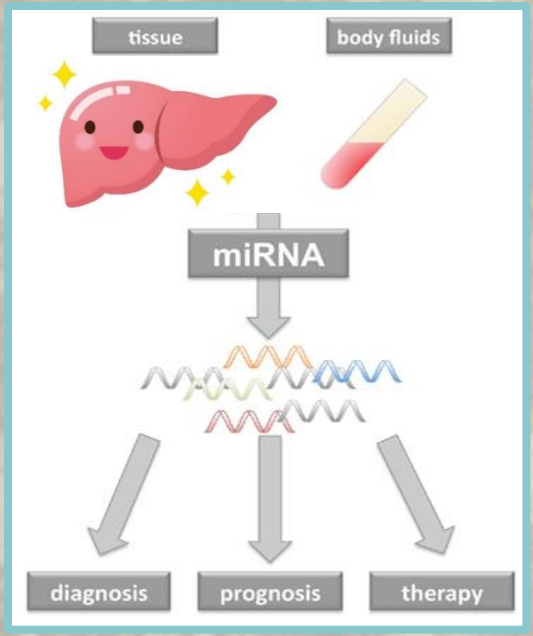


LPI Assay Results over 40 Minutes
(Slopes = ROS activity)

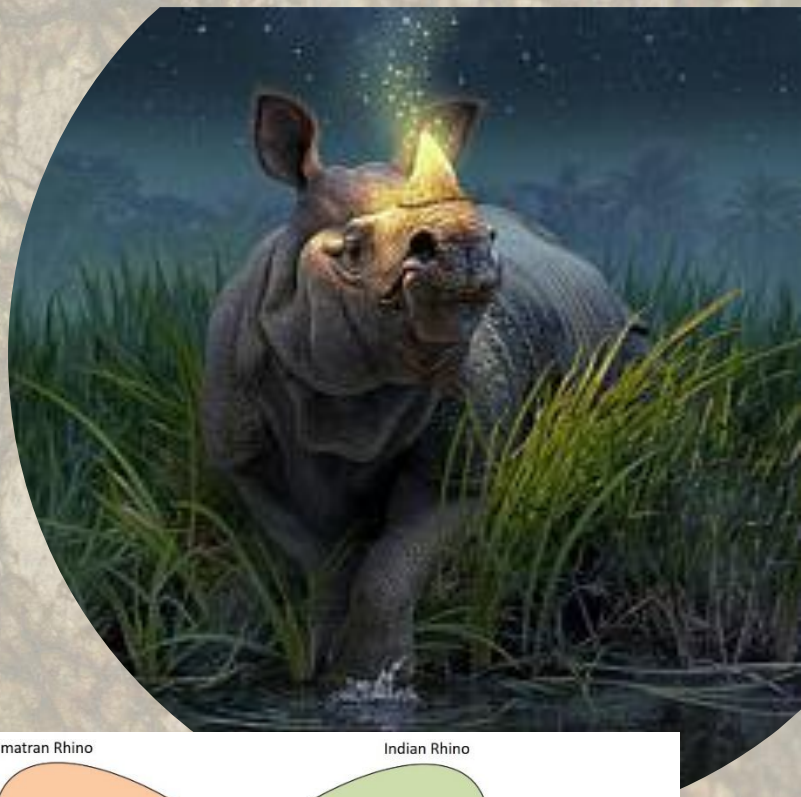
What is causing such extreme ROS activity in black rhino serum?

Rhino Serum microRNAs

(Jessye Wojtusik)



<u>MicroRNAs</u>: Small RNA that can regulate gene expression

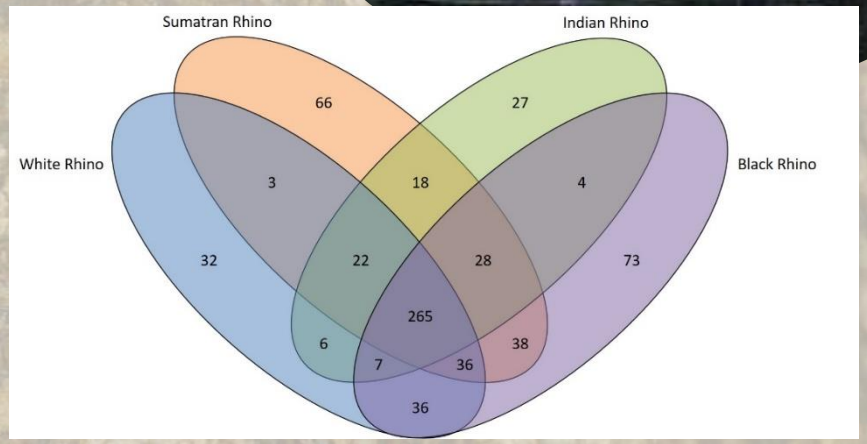


<u>Goals</u>:

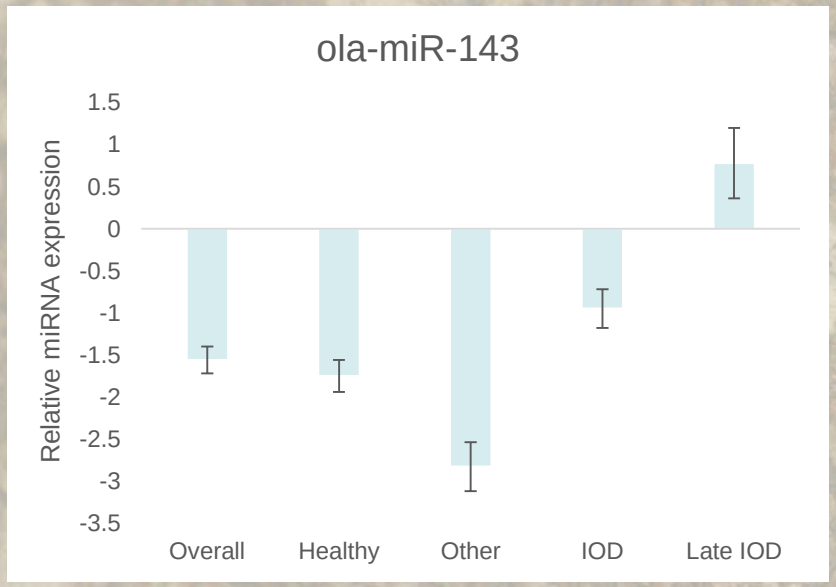
***Identify miRNAs in rhino serum**

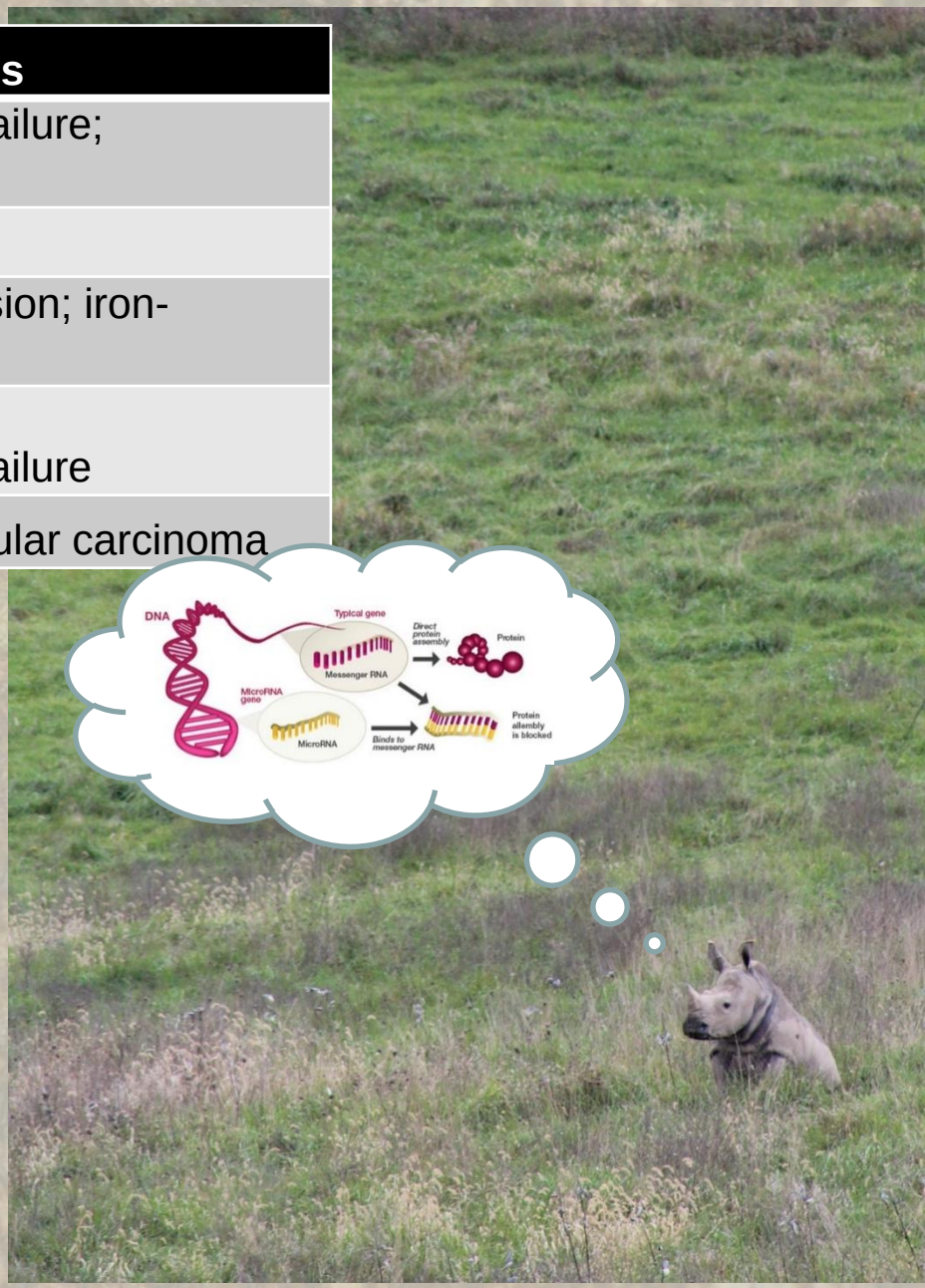
***Determine if any differ between sick and healthy animals**

***Characterize changes over disease progression**



miRNA	Functions and Associations
bta-miR-30e-5p	hepatocyte apoptosis; liver failure; hepatocellular carcinoma
gga-let-7g-5p	tumor suppression; cancer
hsa-miR-146a-5p	hepatic stellate cell suppression; iron-stressed cells
hsa-miR-16b-5p	hepatocyte apoptosis; liver failure
ola-miR-143	abundant in liver; hepatocellular carcinoma





Is the rhinoceros gut microbiome involved?

(CREW, Stanford University, Cincinnati Children's Hospital Medical Center)

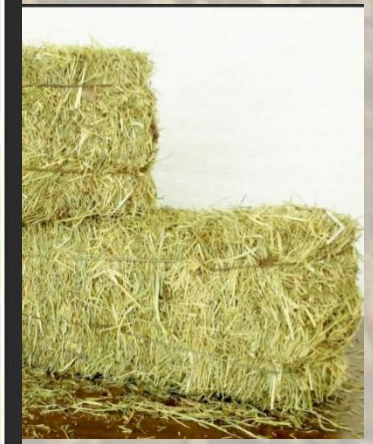
In situ

black rhinos

white rhinos

Proposed Hypothetical Model

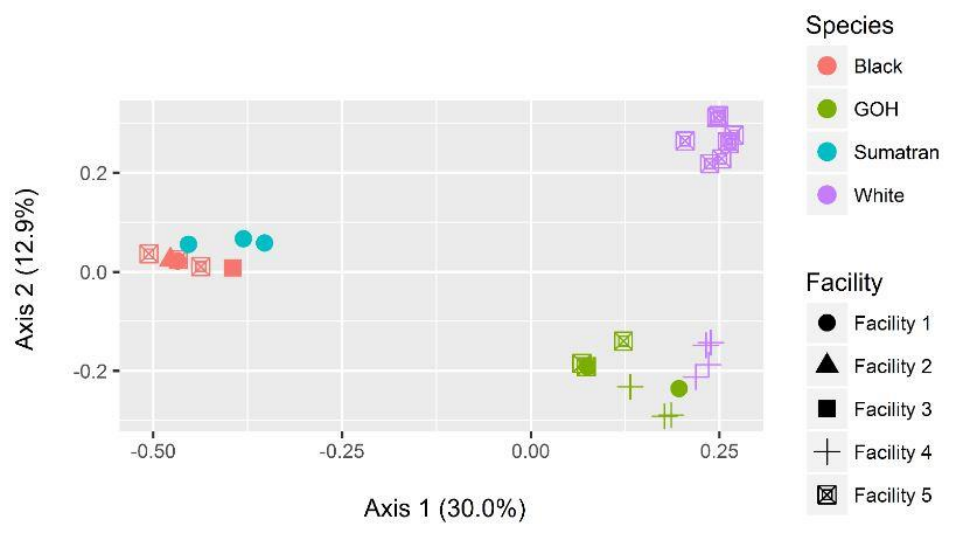
e ex situ

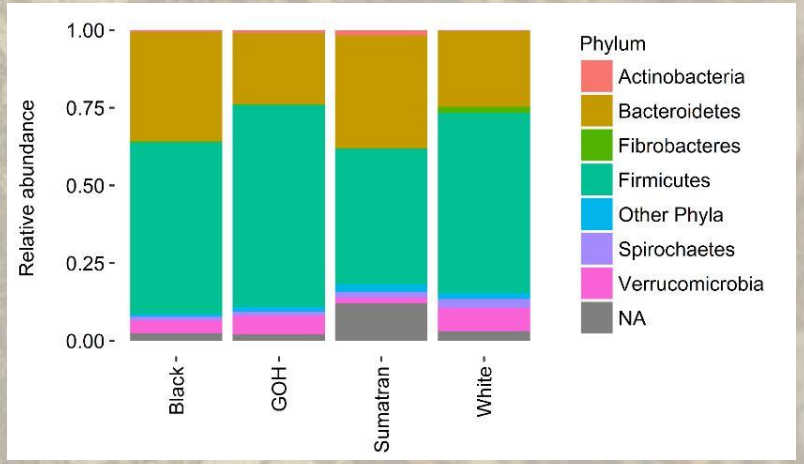


biome

impacted

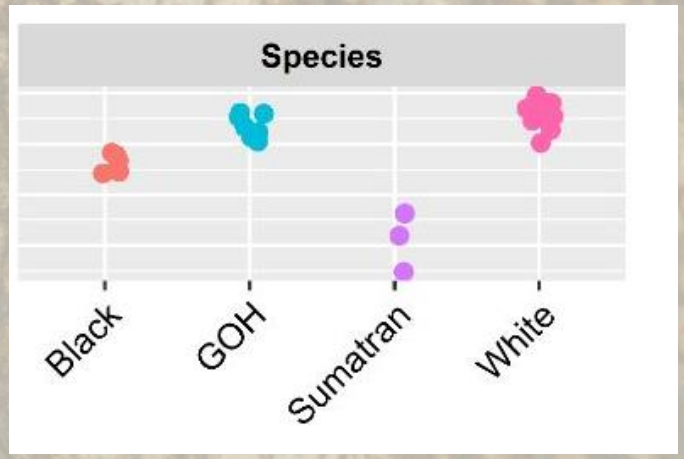
Rhino Microbiome Results





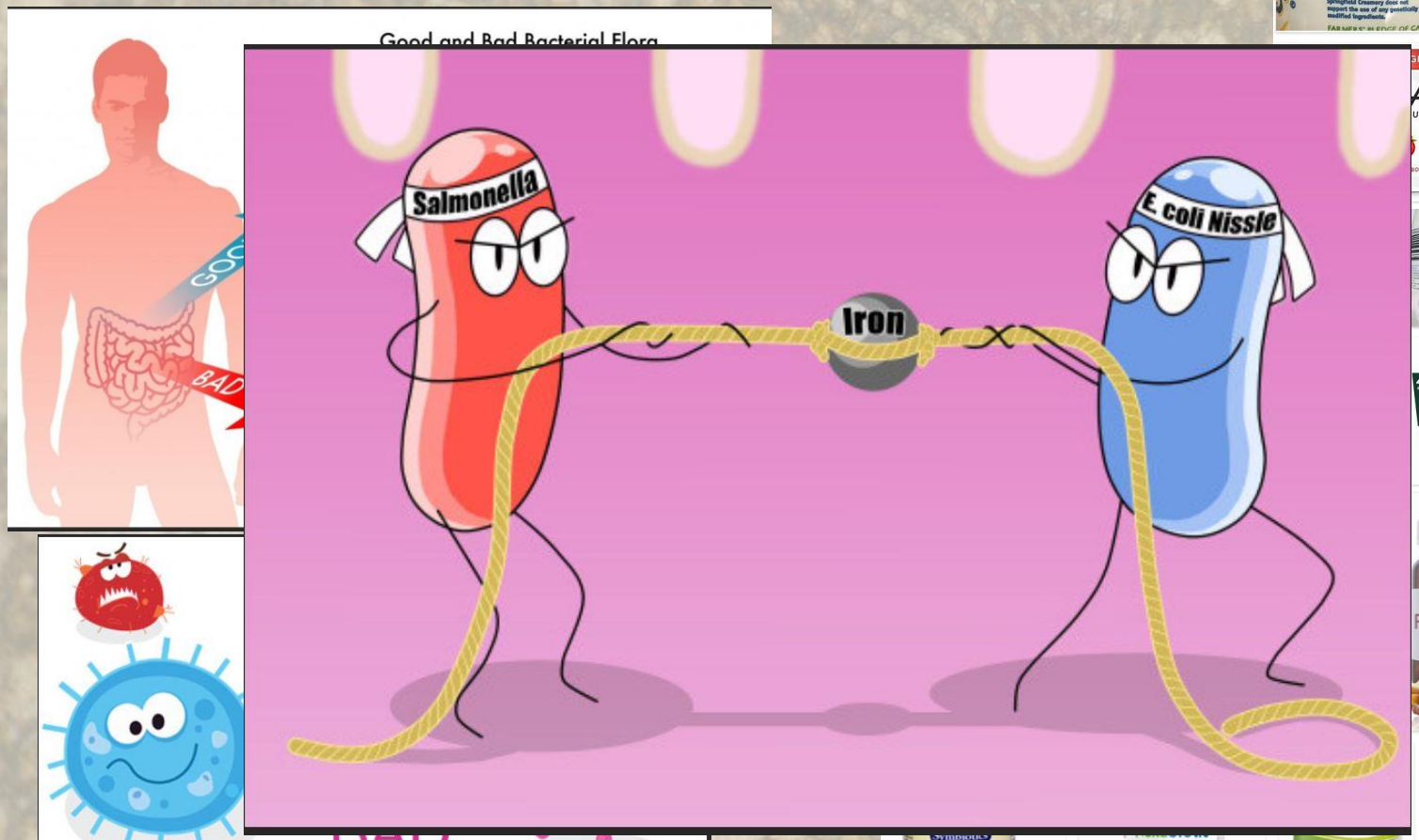
- Black and Sumatran rhinos similar
- Black and Sumatran rhinos lack diversity
- Sex and age were insignificant
- Facility was factor
- **IOD Susceptible/resistant = most significant factor**

Microbiota dviersity

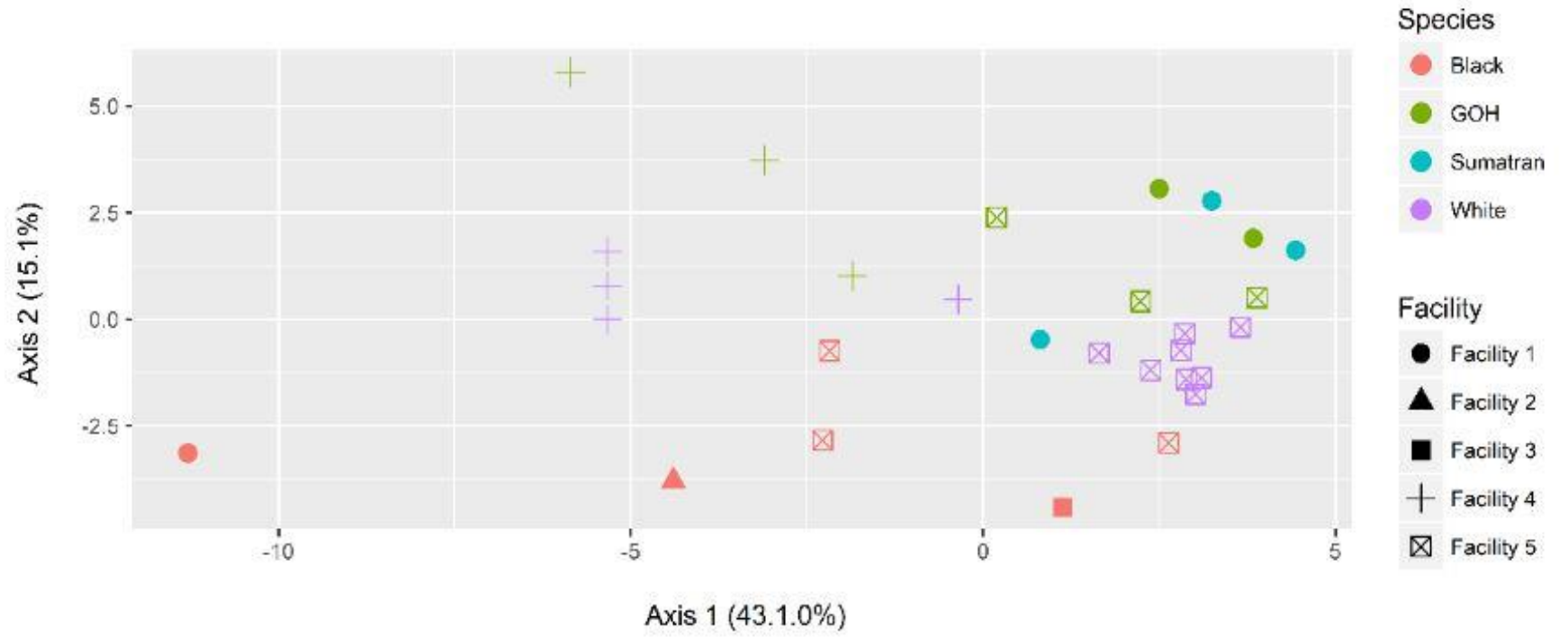


Gut microbial diversity is good!

(as long as the microbial populations are the right kind)



Rhino Metabolome Results



Once again, species differences complicate matters!

Although similarities in gut microbiome between IOD susceptible species suggest an association with IOD, metabolic disturbance may be the more important driver of IOD in black rhinos. Data support findings of Schook et al., in 2015 (Gen Comp Endo).

Black rhinoceros

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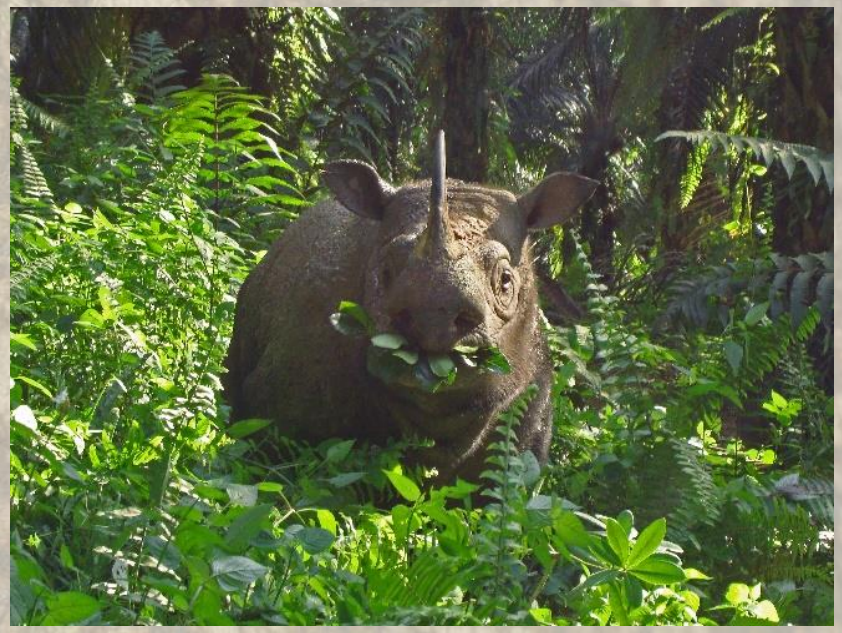
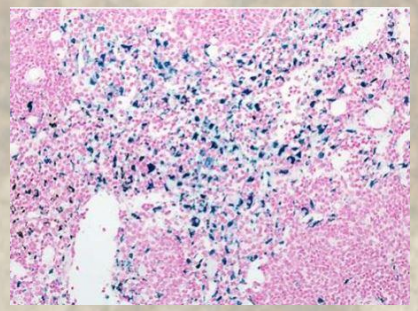
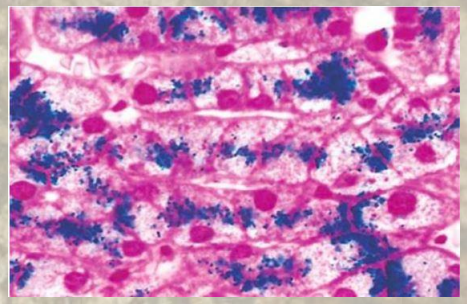


Sumatran rhinoceros

Rhino liver tissue iron study

Dalen Agnew, Mary Duncan, Eric Miller, Michele Miller, Terri Roth



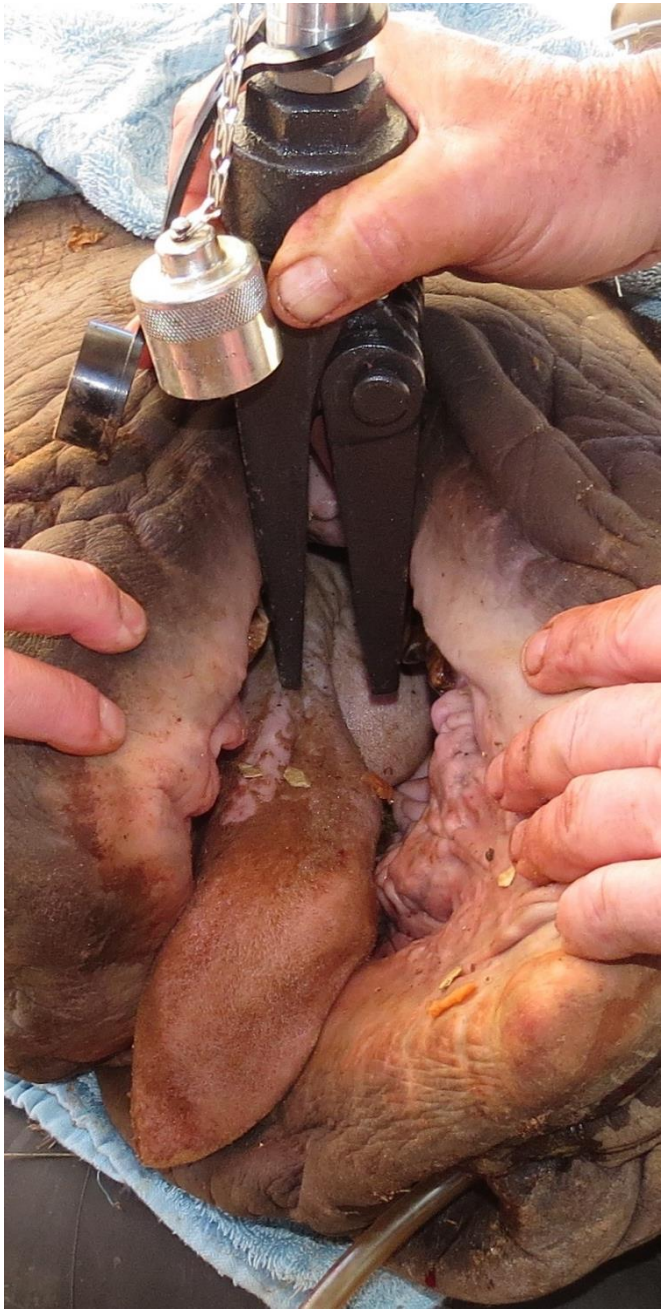


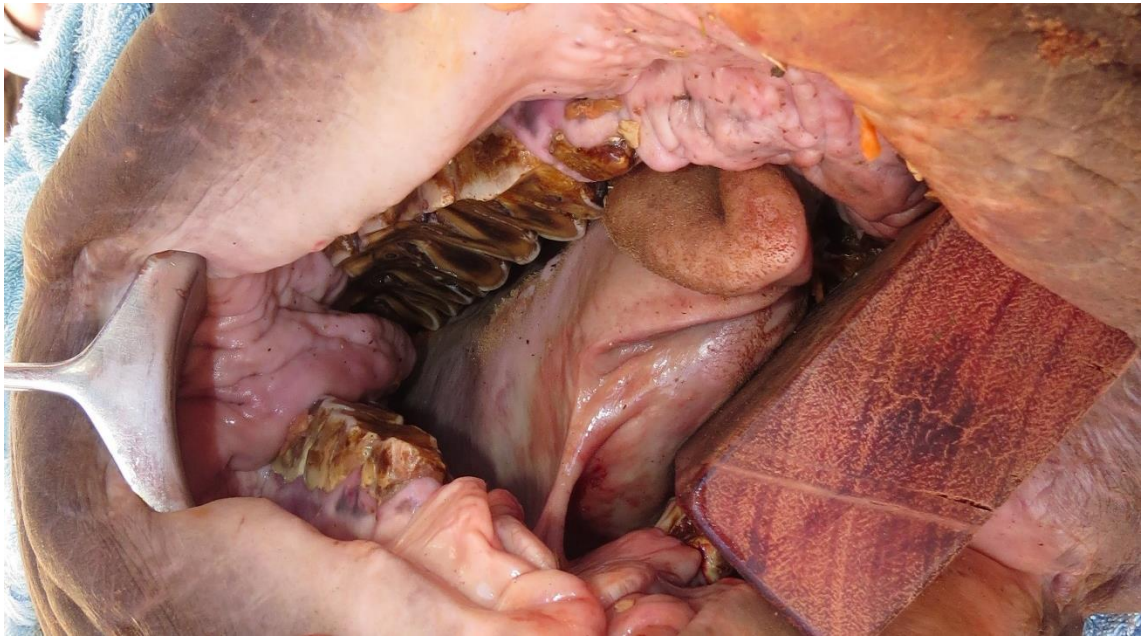
Goals:

- **>60 rhino liver tissue samples to be analyzed for total iron**
- **>20 rhino liver histology slide sets to be reviewed/scored**
- **Two expert pathologist opinions on same slides**

Questions to be answered:

- **Is the iron stored in organ tissues actually damaging the cells/tissue integrity?**
- **How prevalent and how severe is the problem in black rhinos?**
- **Does liver tissue iron content correlate with histopathology scoring?**
- **Is there a trend in necropsy findings that correlate to liver iron scores?**





Dental Disease in Rhinoceroses

Images and information courtesy of Dr. Benn Bryant, Taronga Zoo, Australia

Note Calculus and Gingival Hyperplasia

Dental survey of black rhinos in 2010 indicated 29% (n=16) had received thorough oral exams and 100% of those had abnormalities.

A SURVEY OF DENTAL DISEASE IN CAPTIVE BLACK RHINOCEROSES (*DICEROS BICORNIS*)

Beth Westeren Romig, DVM,[1,2*], Michael Q. Lowder, DVM, MS[1], and Scott B. Citino, DVM, Dipl. ACZ[3].

Periodontal disease is likely much more common in black rhino than is recognized. It has features similar to horses plus:
Hypercementosis = excessive buildup of cementum which surrounds roots. This material is not calculus

Why is this happening? We speculate….

- Browsers eating grass hay (with lots of abrasive silica)
- Years of too much grinding force on the teeth and jaw lead to chronic trauma and periodontal inflammation leading to diastema, food pocketing, gingivitis etc (just like horses) AND hypercementosis
- Chronic, undetected inflammation

Could dental disease be linked to elevated ferritin in black rhinos?

Could it explain elevated inflammatory markers reported by Schook et al., 2015 in zoo-maintained black rhino?