# Saving Species With Science® CREDOR Review Viduation and Research of Endangered Wildlife

# **ROTH'S REMARKS** CREW's New Discovery Station

Although CREW's work saving species with science has achieved international recognition, some of the Cincinnati Zoo & Botanical Garden's daily visitors have never even heard of CREW, much less its progress in safeguarding exceptional plants, rhinos, cats, and polar bears from extinction. CREW's previous Public Exhibit provided a location where Zoo visitors and school groups could catch a glimpse of the conservation science being conducted at our Zoo while a dedicated CREW volunteer explained the work. When CREW added an



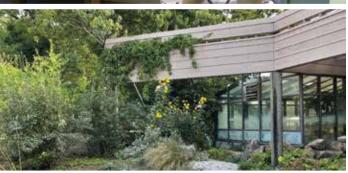
Dr. Terri L. Roth VP of Conservation & Science and Director of CREW

interactive component – Charlotte, the life-size rhino ultrasound model – the visit to CREW took on new energy, and we realized it was time to refresh the entire space and add more new components. The renovation took place over the past year, and we are now proud to announce the completion of the CREW Discovery Station – a gateway into the scientific marvels of CREW. On the way into

CREW, visitors can enjoy the unique, endangered species garden, and once in the Discovery Station, they will learn about CREW's *Signature* Conservation Projects, view the CryoBio-Bank, see first-hand why beautiful cats like ocelots are imperiled, learn how volunteers perform overnight animal watches (and why), witness plant tissue culture in process, get up close to a massive male polar bear (Lord of the melting Arctic) and, of course, perform an ultrasound exam on Charlotte (sans the smell or risk of a living rhino). The Discovery Station is already being utilized by school groups and some of the Zoo's overnight programs. In 2023, it will be open to general zoo visitors when staffed by a volunteer (11 am to 2 pm) from Memorial Day to Labor Day, and on some weekends.









# **RHINO** SIGNATURE **PROJECT**



American Institute of Rhinoceros Science (AIRS): A Model for Saving Species With Science Ex Situ



# CREW is WILD about The Wilds!

CREW rhino scientists have been collaborating with The Wilds for 25 years, but 2022 marked the evolution of an even closer working relationship between the two facilities with the implementation of AIRS. The Wilds offers a unique opportunity to study the most successful rhino breeding center in North America, boasting five generations of rhinos born at the facility. What sets The Wilds apart from most zoos is the management strategy, one that few other locations can replicate. During the winter, rhinos are housed in large, heated barns with access to adjacent paddocks, a system that mimics that used in many zoos. But during the summer months, the entire female crash (i.e., group) along with that year's breeding bull are moved to a 138-acre pasture, complete with hills, wallows, lakes, and trees, allowing up to 18 rhinos to live in a multigenerational, semi-free ranging setting. Studying the physiology, reproduction, and wellbeing of this rhino group under these two extremes in management will be invaluable in gaining insight into the environmental factors that are important for optimizing rhino care, health, and longevity in zoos. A new CREW staff member, Dr. Parker Pennington,

oversees all AIRS activities at The Wilds including monthly and weekly blood sampling, behavior and temperament observations, activity levels, and body mass measurements. Parker received her B.S. and M.S. from Louisiana State University, her Ph.D. from George Mason University, and conducted her post-doctoral training at the San Diego Zoo Global's Institute for Conservation Research where she was integral in the successful production of white rhino calves by artificial insemination.



Dr. Parker Pennington



# Do Rhino Horns Hold Secrets to Rhino Health?

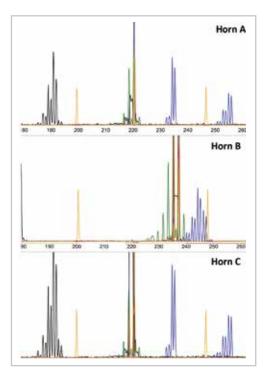
We know that black and Sumatran rhinos store excess iron in their livers, a condition termed iron overload disorder (IOD). but we have no way to monitor just how much they are storing while alive because liver biopsies simply aren't feasible in rhinos. During AIRS brainstorming sessions two years ago, the idea of measuring iron in rhino horns emerged. We know from the scientific literature that human hair (keratin) accumulates different minerals, including iron, that can reflect body concentrations. Since rhino horn is also made of keratin, it may do the same. The first phase of our AIRS IOD study was to test samples taken from different areas along the horn to determine what minerals can be detected and their variation due to location and/or sample color. Results revealed there are many minerals in rhino horn (including iron!), but samples must be taken at least 1 cm deep to avoid environmental contamination. Apparently, all that mud wallowing gives dirt ample opportunity to permeate the horn's surface. With this critical information in hand, we have now initiated the second



phase of the study – determining if black rhino horns contain more iron than white rhino horns. Since white rhinos do not suffer from IOD, their horns should contain lower iron concentrations if rhino horn iron does indeed reflect body iron loads.

# Whose Horn is It Anyway?

So, you are given a box of rhino horns for mineral analysis, but all the identification labels have come off, What do you do next? A similar scenario recently happened at CREW. To answer that question, CREW scientists took on the role of forensic DNA detectives (much like you see on one of the CSI TV shows). With less than a smidgen of horn, CREW researchers isolate DNA and create a rhino's profile by sequencing the DNA at 23 different loci known to differ among rhinos. By partnering with the Cincinnati Museum Center's John A. and Judy Ruthven DNA lab, CREW can generate the DNA profiles quickly (days instead of weeks of time to get the data). Then the scientists play a very advanced game of matching with the assistance of computers, lining up the different DNA profiles. Not only do they identify the species and gender of the individual a horn sample came from; they can determine which horns came from the same individual (note: black and white rhinos have two horns). This can be very challenging if the horns came from related rhinos because siblings share half their DNA. These CSI tools that CREW staff has employed for its work are not just part of your favorite crime show, these are the same tools used to catch and prosecute rhino horn poachers in Africa and are very similar to the process used for ancestry testing.



# Got Milk?

It is common knowledge (and common sense) that a female rhino's udder is expected to enlarge and produce milk near the time of parturition, so her newborn calf has a warm, nutritious meal waiting for it as soon as he/she is born. But what happens when a female starts to produce milk mid-way through a pregnancy? You get very concerned caretakers and very intrigued scientists. For years, CREW has been studying hormones that control animal reproductive function including estrogens, progesterone, testosterone, and several more. However, one hormone that has received little attention in the CREW endocrine lab or rhino community is prolactin the hormone associated with lactation. Recently, CREW was involved with two mid-pregnancy lactation cases in white rhinos which provided a great opportunity to expand our hormone and reproductive knowledge. As a part of AIRS, and to investigate the mid-gestation lactation described above, we began a quest to identify a prolactin assay developed for other species that would work for rhinos. As with most new scientific endeavors, it took several tries before we succeeded, but we are pleased to report that we have found an assay that is a good fit for our needs. Although we currently have more

questions than answers about the strange mid-gestation lactation phenomenon in a few rhinos, we are hopeful this new rhino prolactin assay will be the key to understanding it. Furthermore, it may shed some light on rhino reproductive dysfunction which is an important goal of AIRS.



Credit: The Wilds

# The Black and White of Serum Ferritin in Rhinos: Is There a Connection to Health?

CREW's scientists continue to work on the puzzle that is iron overload disorder (IOD) in black rhinos. Ferritin has been a biomarker of interest because it is the protein responsible for storing iron in the body, but it is also an acute phase protein which means it typically increases in response to illness or inflammation. Ferritin concentrations can be extremely high in the serum of some black rhinos, but black rhinos rarely die from liver failure due to excessive iron stores. Therefore, CREW scientists began to wonder if high ferritin concentrations in rhinos are associated with inflammation rather than iron load. Using previously collected samples stored in CREW's rhino serum bank, CREW scientists analyzed two inflammatory biomarkers and compared their values to those of ferritin. Rarely was an increase in ferritin coincident with a spike in the two other inflammatory biomarkers in either black or white rhinos. This result was somewhat surprising since serum ferritin can be one of the factors used to monitor the onset and recovery of an injury or illness in other mammals. Regardless, the absence of a strong, positive correlation between ferritin and the two inflammatory biomarkers, makes it unlikely that acute inflammation explains the very high ferritin concentrations detected in some black rhino serum samples. Unfortunately, these results do not answer our question about why some black rhinos have exceptionally high ferritin, but by now, we have learned that answers to rhino IOD questions are rarely black and white; instead, they tend to be shades of gray, much like the amazing creatures themselves.



# Piecing Together Panda Pregnancy Puzzles



The Cincinnati Zoo & Botanical Garden boasts an impressive track record of successfully managing red pandas and has produced 98 cubs between 1983 and 2021; however, the overall population in zoological institutions exhibits a much lower success rate. Only 50% of pandas paired for breeding produce cubs, but the timing and reasons for reproductive failure are largely unknown. Insights into the rate and timing of reproductive failure may illuminate potential causes and contributing factors, ultimately allowing for improvements in animal care. To try to better understand pregnancy failure, scientists at CREW analyzed ultrasound videos and images obtained from Cincinnati Zoo pandas over the past 12 years and have detailed the incidence and timing of pregnancy loss. Results showed that 7 of 20 (35%) concepti were lost prior to birth and that the pregnancies failed between days 23-50 pre-partum. Both partial losses (vanishing twin syndrome) and full losses were documented. Recently, it was noted that a new pregnancy was established almost immediately following the loss of the first conceptus, an observation that has never before been described in this species. Results of this analysis provide novel insights into red panda pregnancy and may be relevant to other species, such as polar bears, that experience low reproductive rates.

# POLAR BEAR SIGNATURE PROJECT

# Leaping Lizards (to Study Bears)!

Dr. Emily Virgin is CREW's newest postdoctoral scientist. Born and raised in Illinois, Emily obtained her B.S. in Biology from Northern Illinois University in 2016. Shortly after graduating, Emily began her Ph.D. at Utah State University, where she worked with Dr. Susannah French to study the effects of urbanization on the reproductive physiology and ecology of female side-blotched lizards. While her dissertation research focused primarily on an abundant, desert-dwelling species, Emily sought out opportunities to apply her integrative skillset to on-the-ground conservation efforts. She assisted in physiological and reproductive monitoring of endangered Bahamian rock iguanas exposed to ecotourism and helped collect baseline reproductive information on the elusive Roatán spiny-tailed iguana. Emily defended her Ph.D. in June of 2022 and joined CREW to work on the Polar Bear Signature Project. Through support from IMLS, Emily is working closely with staff scientist Dr. Erin Curry to identify physiological biomarkers in polar bear blood samples that are associated with health and reproduction. Emily's background in physiological ecology brings a unique perspective to CREW's Animal Division, and her expertise will help us better understand polar bear physiology across life-history stages and environmental contexts.



# A Final Foray with Fecal Protein

Several reports have described the identification of specific fecal proteins that may serve as pregnancy biomarkers in wildlife, including polar bears; however, repeatability across labs and by CREW scientists has been limited. In contrast to stable steroid hormones, like progesterone and testosterone, proteins are more fragile structures and can easily break down in suboptimal conditions, like in feces. Inconveniently, most laboratory methods commonly utilized to quantify proteins rely on an antibody's ability to bind to a specific structure on the protein of interest. If the protein is degraded, it is unlikely that the antibody will bind. So, even though the protein might be present in a sample, its degraded state allows it to evade quantification by antibody-based methods of detection. To address this challenge, CREW scientists utilized a new cutting-edge method called label-free quantification (LFQ), which does NOT rely on antibody-binding, hoping that fecal proteins consistently indicating pregnancy in polar bears could finally be identified. We extracted protein from 50 fecal samples collected from pregnant and non-pregnant females, quantified all proteins and protein fragments in the sample using LFQ, and then compared proteins between groups. Unfortunately, results indicated that there was



no protein unique to the pregnant state and that there were no differences in the amounts of specific fecal proteins between pregnant and non-pregnant polar bears. Although not the answer CREW scientists had hoped for, findings highlight the challenges of detecting subtle shifts in physiological biomarkers in wildlife populations.

# **EXCEPTIONAL PLANT** SIGNATURE **PROJECT**

# IMLS Funding for Expansion of Exceptional Plant Research

In August, CREW's Plant Division received the great news that their National Leadership Grant proposal to the Institute of Museum and Library Services had been funded. The project, Advancing the Science of Conservation Cryobiotechnology: Using Oaks as a Model for Improving the In Vitro Technologies Supporting Exceptional Plant Conservation, will continue CREW's work on exceptional species conservation, this time focusing on oak conservation. CREW has shown that shoot tip cryopreservation is a viable option for conserving oak genetic resources, but the first step in that process-initiating healthy oak shoot cultures-is often difficult and unpredictable. This study involves collaboration with arboreta and collectors from around the country to examine the effects of species, genotype, stage of growth, and environmental factors on the initiation process over three collecting seasons. Different types of cultures will be compared, and a University of Cincinnati graduate student will be supported to study any genetic changes that occur during initiation and growth of those cultures. In addition, funds are provided to enlist five additional labs to examine factors affecting oak culture





initiation, thereby increasing the study's data while expanding and developing expertise in oak culture. Finally, the project will build on our previous grant's work to update and expand the List of Exceptional Plants (https://cincinnatizoo.org/ global-list-of-threatened-exceptional-plants/) and to develop new online tools to help researchers working with these species. It's an ambitious project, but it will be exciting to see what can be learned over the next three years!

# People Were DYING to See Morticia

When the Zoo's corpse flower (Amorphophallus titanum), Morticia, finally bloomed in July, CREW plant scientists were on hand to collect valuable data. Corpse flowers have the largest inflorescences in the world, and take 5-7 years of growth to bloom. After days spent watching her, the Plant Division got the call that she was blooming late on a Friday night and raced in to set up the infrared camera to get a thermal time-lapse of the bloom. Whereas the plant deceivingly looks like one enormous flower, the actual flowers are very small and numerous, located near the base of the tall central spadix. The plant has separate male and female flowers that bloom at different times, making self-pollination impossible. Because she's primarily pollinated by flies, Morticia emitted a strong scent of decaying flesh when her flowers were at their peak to attract carrion insects for pollination. To advertise her flowers far and wide, the spadix heated up to over 30°C (86°F) around peak bloom! In addition to tracking her temperature, we also helped the Horticulture team collect pollen for future pollination events. Most gardens with corpse flowers store the pollen at -20°C (in a conventional freezer), but we also took the opportunity to cryopreserve the corpse flower pollen in liquid nitrogen to extend its longevity. Thanks to CREW's work, we have a stash of pollen in the freezer and the CryoBio-Bank for the next time Morticia blooms - 7-10 years from now!





# Schmidlapp Scholar Rooting for the Recovery of a Threatened Species

CREW Plant Research Division's Schmidlapp Scholar Gillian Ross recently finished up an impressive internship with two completed projects on plant conservation! After earning her M.S. degree in Conservation Genetics from Bowling Green State University, Gillian turned her focus to the federally threatened Lakeside daisy (Tetraneuris herbaceae). The only wild US population of this species is found on the shores of Lake Erie in Ohio, but nearly the entire population is located in Lafarge Quarry, an active limestone quarry that is regularly blasted for mining. As a result, the Ohio Department of Natural Resources (ODNR) has worked for years to collect seeds from the wild populations and move them to protected areas on nearby Kelleys Island to preserve the species. However, the Lakeside daisy is genetically self-incompatible, meaning that it has a genetic mechanism that prevents flowers from being pollinated by too closely related relatives. This makes the preservation of genetic diversity in the species of the utmost importance to its survival. Using amplified fragment-length polymorphism (AFLP) markers, Gillian was able to show that the wild and relocated populations were genetically indistinct from each other, meaning that ODNR is doing a great job at mimicking the wild population in their protected relocation. However, overall genetic diversity was very low, which causes concern about the ability of the species to produce seeds and persist long-term. After her internship, Gillian accepted a position at the Toledo Zoo as a Wild Toledo Biologist working on butterfly research and conservation. Congratulations, Gillian!



# Don't Doubt DNA

## The Secret to Effective Conservation Strategies Could be Hidden in the Code!

Genetic analyses are currently center-stage for many biological disciplines, especially conservation biology. Rachel Bridgens, a CREW plant lab graduate student, is using genetics to understand the diversity remaining in endangered species and how to effectively restore populations. *Gardenia brighamii* is a critically endangered Hawaiian woody shrub with only 15 individuals remaining in the wild! Unfortunately, *G. brighamii* 

is one of many Hawaiian plants with such low population numbers as a result of many anthropological alterations such as the introduction of invasive species, population fragmentation, habitat encroachment, etc. Considering the extremely small population sizes and seemingly limited genetic diversity, are these species even salvageable? Rachel worked to answer this intense question in our recent IMLS-funded project by investigating the genetic diversity remaining in wild popu-



lations of *G. brighamii* and comparing it to that of the diversity stored in Lyon Arboretum's Micropropagation Laboratory. She compared DNA from wild- and ex situ- collected plant tissue and found that the ex situ population stored at Lyon Arboretum was representative of the three wild populations located on the island of Lana'i. In fact, the ex situ population, collected from 2005-2012, con-

tained genotypes that have since been lost from the wild populations. These genetic analyses provide critical information for managing species' conservation and help guide the prioritization and allocation of resources to species that are genetically more likely to survive. Rachel is currently finishing up her M.S. degree and will become the DNA Lab Manager at the Cincinnati Museum Center later this summer. Congratulations, Rachel!

## **IMPERILED CAT** SIGNATURE **PROJECT**

## Doubling Down on Cat Welfare



For the millions of cats that enter animal shelters each year, housing is a constant and ongoing experience that impacts their health and welfare. Shelters face a trade-off between maximizing the number of spots available and providing adequate space for each cat. Double compartment housing (i.e., cages with two separate housing areas connected by a portal) has been proposed as one approach to provide cats with more livable space, but little research has quantified its possible welfare benefits. To address this knowledge gap, Dr. Julie

Barnes, a veterinarian who joined CREW as a post-doctoral scientist three years ago, recently completed a novel cat housing study. Julie sought to answer this welfare question utilizing automated technologies: Fitbits to quantify cat activity and radio frequency identification (RFID) tags to track location. These autonomous data collection methods reduced the intense time commitment previously required and enabled compiling wider and larger data sets, including 24-hr monitoring. The study was performed at three local Cincinnati shelters to compare activity profiles, location patterns, and stress levels (via fecal glucocorticoid analysis) of cats housed in either single or double compartment layouts. Results indicated that cats in double compartment housing spent significantly less time in their litterboxes and showed decreasing glucocorticoid levels. This study was one component of Julie's graduate research program at the University of Cincinnati where she was awarded her M.S. degree this past June. Julie plans to remain at CREW and is currently exploring her options for pursuing a Ph.D. focused on wild cat species. (This study was funded by the Joanie Bernard Foundation.)

# Every Meow You Take - We'll Be Watching (Over) You

CREW has made tremendous strides in helping to develop a gene therapy-based approach for non-surgical sterilization of female domestic cats. Although the cats, CREW staff, and our collaborators deserve much of the credit, the unsung heroes in this research success are dozens of CREW volunteers who literally have kept their eyes on our cats every step of the way. Each day, volunteers provide loving care to CREW cats, ensuring that they receive proper nutrition, and live together in an enriched, healthy environment. By observing the cats closely, our volunteers can tell which cats are having a good day or which ones might need a friendly snuggle or a veterinary check-up. Within each cat room, digital infrared cameras allow 24-hour access to monitor cat activity, including behaviors during breeding trials designed to assess sterilization effectiveness. For each trial, a male cat is housed with a group of females for four consecutive months (40 hours/week) and every breeding attempt is noted by Zoo Volunteer Observers (ZVOs) who review all digital video recordings. These behavioral assessments allow us to document female estrous activity and identify mated females for follow-up ultrasound-based pregnancy exams. Pregnant females are transferred into a

separate maternity room equipped with additional cameras where they can be monitored remotely 24/7 for signs of labor and impending parturition, at which point staff are notified and arrive at CREW in case queens or newborn kittens need any assistance. For the sterilization studies alone, ZVOs donated almost 2000 hours of observation time to review breeding behavior and monitor the birth of eight litters. The dedicated assistance of our animal care volunteers and ZVOs helped make this scientific breakthrough possible – they really are the cat's meow!



# FRIENDS OF CREW

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# SCIENTIFIC HIGHLIGHTS

## **CREW WISH LIST**

**PHOTOBOX** - This item will be used by the Plant Division to take high-quality photos of cultures and experiments for use in more advanced analyses. **Cost: \$50.** 

**HEAT BLOCK/TUBE HEATER** - This heating device keeps small tubes and their contents warm and is a huge help when processing sperm samples. **Cost: \$100.** 

**MAGLITE** – Have you ever tried to look into a liquid nitrogen tank? CREW scientists do regularly and a good flashlight is key. **Cost: \$100.** 

SMALL MANUAL DEFROST FREEZER – For storing molecular reagents that are sensitive to thawing during automatic defrost cycles. Cost: \$350.

**MAGNETIC STIR PLATE** – Essential for making buffers in the lab, especially when reagents don't readily dissolve. **Cost: \$399.** 

**VORTEX MIXER** – Used daily for mixing solutions. Our current vortex is showing its age. **Cost: \$500.** 

**DRY ICE MAKER** – This new item will help us to inventory and sort thousands of rhino samples that will be arriving at CREW for the AIRS projects without having to risk hypothermia in the walk-in freezer. **Cost: \$1,200.** 

**CHLOROPHYLL FLUOROMETER** – This handheld device can monitor photosynthesis activity in plant tissues and will be used by the Plant Division for early and accurate quantification of plant stress. **Cost: \$3,000.** 

**MULTI-TUBE VORTEXER** – This mixer would greatly reduce staff time required to process blood samples from rhinos and polar bears for analysis. **Cost: \$3,700.** 

**MICROBALANCE** – Used by the Plant Team to measure small quantities of hormones and nutrients for tissue culture media. The current microbalance is over 30 years old and starting to malfunction. **Cost: \$5,000.** 

## **BOOK CHAPTERS**

**Curry E**. 2021. Reproductive biology of the red panda. In: Angela R Glatston (ed), Red Panda: Biology and Conservation of the First Panda, 2nd Ed. London, UK: Elsevier, Pp. 119-138.

Vansandt LM. 2022. Feline estrous cycle. In: Johnson A and M Kutzler (eds). Feline Reproduction. Boston, MA: CAB International, Pp. 11-22.

Swanson WF. 2022. Chapter 28: Application of assisted reproductive technology in non-domestic felids. In: Johnson A and M Kutzler (eds). Feline Reproduction. Boston, MA: CAB International, Pp. 269-278.

Swanson WF. Chapter 108: Semen banking of wild felids for zoo vets. In: Miller RE, N Lamberski and P Calle (eds). Fowler's Zoo and Wild Animal Medicine Current Therapy, Volume 10. St. Louis, MO: Elsevier, Pp. 773-780.

### PEER-REVIEWED PUBLICATIONS

Blank MH, CH Adania, **WF Swanson**, DSR Angrimani, M Nichi, MABV Guimaraes and RC Barnabe. 2022. Comparative fecal steroid profile during pregnancy, parturition, and lactation between natural fertilization and embryo transfer in ocelots (*Leopardus pardalis*). Theriogenology 182:26-34.

**Curry E, M Philpott, J Wojtusik,** WD Haffe, MA Wyder, KD Greis and **TL Roth**. 2022. Label-free quantification (LFQ) of fecal proteins for potential pregnancy detection in polar bears. Life 12:796, https://doi.org/10.3390/life12060796.

**Donelan EM, M Philpott, KM MacKinnon, KA Klosterman** and **TL Roth**. 2022. Faecal glucocorticoid metabolite concentrations associated with illness, sex, age, and season in a kea *Nestor notabilis* population at the Cincinnati Zoo & Botanical Garden. Journal of Zoo and Aquarium Research 10(2):107-114, doi: 10.19227/jzar.v10i2.654.

**Lowe J** and **E Curry**. 2021. Incidence of pregnancy loss and characterization of fetal development in red pandas. Reproduction & Fertility 2:292-299.

**Pence VC**, E Beckman, A Meyer, HW Pritchard, M Westwood, J Linsky, J Gratzfeld, S Helm-Wallace, U Liu, M Rivers and E Beech. 2022. Gap analysis of exceptional species – Using a global list of exceptional plants to expand strategic *ex situ* conservation action beyond conventional seed banking. Biological Conservation 266, https://doi.org/10.1016/j.biocon.2021.109439.

### **GRANTS AWARDED**

Funding Source: Institute of Museum and Library Services. Project: Advancing the science of conservation cryobiotechnology: Using oaks as a model for improving the in vitro technologies supporting exceptional plant conservation. Role: Principal investigator. Duration: 9/1/22-8/31/25. Amount: **\$399,494.** 

Funding Source: Michelson Found Animals Foundation. Project: Vectored contraception of domestic cats. Role: Principal investigator. Duration: Jan 2022 – Dec 2022. Amount: **\$80,000**.

Funding Source: Global Botanic Gardens Fund. Project: Advancing the ex situ conservation of cycads: Evaluating the requirements for somatic embryo initiation from leaf tissue. Role: Principal Investigator. Duration: 1/1/22 – 12/31/22. Amount: **\$2,186**.

**Pence VC** and EB Bruns. 2022. The tip of the iceberg : Cryopreservation needs for meeting the challenge of exceptional plant conservation. Plants 11:1528 https://doi. org/10.3390/plants11121528.

**Pence VC,** A Meyer, J Linsky, J Gratzfeld, HW Pritchard, M Westwood and EB Bruns. 2022. Defining exceptional species – A conceptual framework to expand and advance ex situ conservation of plant diversity beyond conventional seed banking. Biological Conservation 266, https://doi.org/10.1016/j.biocon.2021.109440.

Roth TL, M Philpott and J Wojtusik. 2022. Rhinoceros serum labile plasma iron and associated redox potential: interspecific variation, sex bias and iron overload disorder disconnect. Conservation Physiology 10(1), https://doi.org/10.1093/conphys/coac025.

Wojtusik J, E Curry and TL Roth. 2021. Rhinoceros serum microRNAs: Identification, characterization, and evaluation of potential iron overload biomarkers. Frontiers in Veterinary Science 8:711576, https://doi.org/10.3389/fvets.2021.71157.

Wojtusik J, E Curry and TL Roth. 2021. Rhinoceros serum microRNAs. Mendeley Data, V4, doi: 10.17632/9wxcd3t3tt.4.

**Wojtusik J**, **TL Roth** and **E Curry**. 2022. Case studies in polar bear (*Ursus maritimus*) sperm collection and cryopreservation techniques. Animals 12:430, https://doi.org/10.3390/ani12040430.

## SCIENTIFIC PRESENTATIONS

**Barnes J, L Vansandt** and **W Swanson.** 2022. Low dose local infusion of dexmedetomidine and spermatic cord traction may improve sperm recovery with urethral catheterization in domestic cats. International Symposium on Canine and Feline Reproduction, Milan, Italy. Oral presentation.

Brandhuber M, S Atkinson, **E Curry** and **TL Roth.** 2021. Investigating a novel biomarker for monitoring reproduction in polar bears. 27th International Conference on Bear Research and Management. Virtual poster presentation.

Brandhuber M, **E Curry**, C Cunningham and S Atkinson. 2022. Determining reproductive success in polar bears using a novel biomarker. Alaska Marine Science Symposium. Virtual platform presentation.

Bridgens R, M Philpott, T Culley, V Pence and S Ching. 2022. Genetic evaluation of the critically endangered woody species *Gardenia brighamii* and hybridization with ornamental *Gardenia tahitensis* in the Hawaiian Islands. Hybrid Botanical Society of America Annual Meeting, Anchorage, AK. Oral presentation.

Carroll R, LA Lyons, **W Swanson**, T Boyd, A Chaney, M Foley, K Terio and WC Warren. 2022. Discovery of transitional cell carcinoma (TCC) candidate risk genes in fishing cats (*Prionailurus viverrinus*) managed within North American zoos. Annual Conference of the Association of Zoos & Aquariums, Baltimore, MD. Poster presentation.

**Curry E, J Wojtusik** and **TL Roth**. 2022. Evaluation of an antibody-free approach to identifying faecal peptides for pregnancy detection in polar bears (*Ursus maritimus*). Reproduction, Fertility and Development 34(2) 246. Proceedings of the International Embryo Transfer Society 48th Annual Conference. Platform and poster presentations.

**Esparza-Harris KC, WF Swanson** and **LA Rispoli.** 2021. Characterization of Toll-Like Receptors 7 and 8 for the sex sorting of X and Y-bearing sperm in domestic cats *(Felis silvestris catus)*. Annual Meeting of the Society for Study of Reproduction, St. Louis, MO. Poster presentation.

Nagykery N, **L Vansandt**, M Kano, D Wang, HD Saatcioglu, M Meinsohn, **A Miller**, **R González**, **J Barnes**, G Guangping, P Donahoe, **W Swanson** and D Pépin. 2022. Evaluation of a second-generation AAV9-MIS vectored contraceptive in cats. International Symposium on Canine and Feline Reproduction, Milan, Italy. Oral presentation.

**Pence VC.** 2022. Defining exceptional plants and how we got here. Conserving Exceptional Plants Virtual Symposium/Workshop. Oral presentation.

**Pence VC.** 2022. The potential of shoot tip cryopreservation as a conservation tool for oaks (*Quercus spp.*). Conserving Exceptional Plants Virtual Symposium/Workshop. Oral presentation.

**Pence VC** and Bruns EBB. 2022. Addressing the needs for conserving threatened exceptional species–The critical role of cryopreservation. CRYO2022, Dublin, Ireland. Invited oral presentation.

**Pence VC** and **Ross G.** 2022. Shoot tip cryopreservation for conserving oak biodiversity ex situ. 10th International Oak Society Conference, Las Cruces, New Mexico. Oral presentation.

**Philpott M.** 2021. Issues in collection of clonal tissues for ex situ conservation. Conserving Exceptional Plants Virtual Symposium. Virtual oral presentation.

**Philpott M** and **V Pence.** 2022. Conservation of exceptional plant species: What does it cost and what do we need? Botanical Society of America Hybrid Annual Meeting, Anchorage, AK. Virtual oral presentation.

Reeves AM, **WF Swanson**, T Campbell, CD Hilton, HM Swarts, T deMaar and DL Miller. 2022. Laparoscopic oviductal artificial insemination with frozen semen for conservation management of endangered ocelots in southern Texas. Annual Meeting of the Wildlife Disease Association, Madison, WI. Oral presentation. **Rispoli LA** and **TL Roth.** 2021. Toll-Like Receptors 7 and 8 and their potential for sex-sorting rhinoceros sperm. Annual Meeting of the Society for Study of Reproduction, St. Louis, MO. Poster presentation.

**Ross G** and **M Philpott.** 2022. Quantification of genetic diversity of Ohio's lakeside daisy using amplified fragment length polymorphism molecular markers. Botanical Society of America Hybrid Annual Meeting, Anchorage, AK. Poster presentation.

Rzucidlo CL, **E Curry** and MR Shero. 2022. Validation of infrared thermography for non-invasive assessment of animal vital rates across wildlife species. International Council for the Exploration of the Sea North / Pacific Marine Science Organization Early Career Scientist Conference. Poster presentation.

**Swanson WF.** 2022. The challenge of assisted reproduction for conservation of wild felids - a reality check. International Symposium on Canine and Feline Reproduction, Milan, Italy. Invited oral presentation.

Swanson WF, HL Bateman, J Newsom, LA Lyons and CA Lambo. 2022. Soy lecithin produces superior fertility compared to egg yolk following semen cryopreservation and laparoscopic oviductal artificial insemination (LO-AI) in domestic cats. International Congress of Animal Reproduction, Bologna, Italy. Poster presentation.

Tompros A, J Wojtusik, M Philpott, TL Roth, M Campbell and E Curry. 2022. Anti-Müllerian hormone in polar bears (*Ursus maritimus*): assay validation and concentrations in relation to sex, age, and season. Reproduction, Fertility and Development 34(2) 245-246. Proceedings of the International Embryo Transfer Society 48th Annual Conference. Poster presentation.

Vansandt L, N Nagykery, A Miller, R González, J Barnes, A Thompson, J Newsom, M Meinsohn, N Sicher, A Kashiwagi, G Gao, D Wang, P Donahoe, D Pépin and W Swanson. 2022. Gene therapy-induced overexpression of Mullerian inhibiting substance (MIS) produces long-term contraception in female domestic cats. International Symposium on Canine and Feline Reproduction, Milan, Italy. Oral presentation.

**Winkeljohn M.** 2021. The role of media formulation and the survival and growth of shoots *in vitro*. Conserving Exceptional Plants Virtual Symposium. Virtual oral presentation.

**Winkeljohn M.** 2022. Creating a stress-free culture: Improving shoot survival *in vitro* through ethylene inhibition. International Oak Society Conference, Las Cruces, NM. Oral presentation.

Winkeljohn M, V Pence and T Culley. 2022. Learning to de-stress: Inhibiting ethylene to improve oak survival *in vitro*. Botanical Society of America Annual Meeting, Anchorage, AK. Oral presentation.

**Wojtusik J, TL Roth** and **E Curry.** 2022. Evaluation of polar bear (*Ursus maritimus*) sperm collection and cryopreservation techniques. Reproduction, Fertility and Development 34(2) 247. Proceedings of the International Embryo Transfer Society 48th Annual Conference. Poster presentation.



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