Saving Species With Science®

CREW ReView

Lindner Center for Conservation and Research of Endangered Wildlife

Cincinnati Zoo & Botanical Garden • Fall 2018

Roth's Remarks

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

Voluntarily Making A Huge Impact

In 1996, when I interviewed for the CREW Director job, I spoke with a number of people in important positions associated with the Cincinnati Zoo & Botanical Garden including the Zoo's Director, the Zoo's Education Director (today's Zoo Director), the Institutional Animal Care and Use Committee Chair, prominent professors at the University of Cincinnati and Medical College associated with CREW, and the CREW Volunteer Chair (yes, really). Volunteers have always been an integral and vital part of CREW, so much so, that the opinion of the CREW Volunteer Chair was solicited in the hiring of a new CREW Director. That individual still volunteers at CREW today, and I often thank her for my job. The importance of the more than 130 volunteers at CREW has never wavered. These generous, talented individuals do everything from greeting guests at the front desk to socializing (and caring for) cats in the colony, from processing animal dung to making sterile media, and from assisting in surgery to designing scientific studies. In addition, there are others working as educators introducing students to CREW's work and talking to visitors at the CREW Public Exhibit and/or CREW cart each year. In fact, over the past five years, CREW volunteers have taught >7,000 students and introduced >280,000 visitors to CREW's research. During that same time frame, 69,305 volunteer hours were logged in, supporting CREW in too many ways to mention, with an economic value of $1,733,708. Perhaps just as important, these generous individuals are also dear friends and valuable colleagues, keeping CREW staff grounded while working side-by-side with us to advance our mission of *Saving Species With Science®*.

Enhancing Ovarian Stimulation Techniques in Polar Bears: Hitting it Right on the Nose

Scientists at CREW are noticing a trend in which female polar bears housed with males tend to exhibit better responses to hormone injections than those housed without males. Because most females that are candidates for AI procedures are not typically housed with males, CREW scientists wondered if providing olfactory stimulation to solo-housed females may serve as a substitute for the presence of a mature male, thereby augmenting current estrus induction protocols. Polar bears have an outstanding sense of smell, so they probably can infer a considerable amount of information by sniffing the scent of another individual. During the 2018 breeding season, fecal samples and urine-soaked straw were collected from Cincinnati Zoo's male polar bear "Little One" and shipped out 2-3x/week for presentation to two females scheduled for AI at other zoos. Although the females did not overtly respond to the initial presentation of the "Odeur de Little One," after receiving their first hormone injections, both females exhibited flehmen responses to the materials, indicating that they were particularly interested in the aromas. Additionally, fecal hormone analysis revealed that both experienced higher concentrations of the hormone associated with ovarian activity than they had the previous year when they were treated with similar stimulation protocols but without olfactory stimulation. Although polar bears are considered induced ovulators, the impact of male presence on ovarian dynamics is unknown in this species. These were the first attempts by CREW to enhance an estrus induction protocol with olfactory stimulation in polar bears and likely will be repeated in future AI endeavors.

Taking Tally of Frozen Lions and Rhinos and Bears (and more!)

In 1982, the first sperm sample cryopreserved by CREW scientists was placed in a liquid nitrogen tank for long-term storage at -196°C. Over the years, additional samples collected by numerous scientists have been added, representing a myriad of gamete collections, late-night post-mortem gamete rescues, post-doctoral research projects, and assisted reproduction efforts. As the number of samples increased, so did the number of storage tanks, which collectively came to be known as CREW's CryoBioBank®. This reserve of frozen genetic material from threatened and endangered species is one of just a few of its kind in the world. By 2017, metal canes containing thousands of samples were packed like sardines within twelve large storage tanks. Due to a need to create more space for incoming samples and to update the electronic inventory, efforts were initiated to physically catalog 35 years-worth of samples, cane by cane. Now the numbers are in: the CryoBioBank® contains over 1500 canes holding sperm, oocytes, embryos, or other tissues from 87 different animal species and subspecies! The repository is dominated by felids with 29 cat species represented, followed by ruminants (23 species) such as bongos, giant eland, and okapi. Rounding out CREW's *Signature* Project animal species are rhinos (4 species) and bears (3 species). Additionally, there are amphibians, primates, otters, and many miscellaneous critters like aardvarks, penguins, and anteaters. Most of these individual animals are no longer living, so storing their genetic material provides some insurance against loss of genetic diversity and options for producing future offspring. *(CREW thanks Praxair, Inc. for generously donating the liquid nitrogen required to maintain the CryoBioBank®.)*

Pavlov Would be Proud!

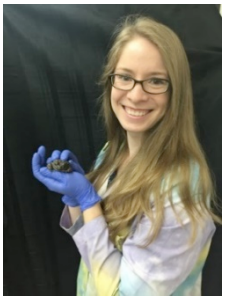
Monitoring hormones to track reproductive cycles or diagnose pregnancy is a common endeavor of animal scientists. To date, poop is the reigning favorite biological sample to work with: it's generally easy to collect, non-invasive, plentiful and easily stored. However, using fecal matter to monitor hormones in the common hippo is difficult due to their preferred method of disposal, best described as the 'helicopter tail'. The sample is swiftly showered in every direction imaginable as it is excreted. This challenge is compounded when hippos are housed in groups, rendering source identification impossible. As an alternative, CREW enlisted help from the animal care team to develop a method to safely collect saliva from the open mouth of hippos using a syringe attached to a long flexible tube and dedicated positive reinforcement training. Samples were collected from seven female hippos at Disney's Animal Kingdom, Memphis Zoo, and our very own Cincinnati Zoo. CREW scientists extracted hormones from the saliva and have shown that results are comparable to those obtained from analyzing fecal samples. Salivary hormone monitoring provides scientists insight into the reproductive status of the common hippo while avoiding the dreaded poop shower, truly a win-win solution. (*Project supported by a gift from The Reuben Herzfeld Fund of the Greater Cincinnati Foundation.*)

Meet CREW's 2018 Charlotte R. Schmidlapp Scholars

ENNOBLE • EMPOWER • UPLIFT
SERVING WOMEN & GIRLS SINCE 1908
THE CHARLOTTE R. SCHMIDLAPP FUND
Fifth Third Bank, Trustee

Thanks to a generous grant awarded to CREW from The Charlotte R. Schmidlapp Fund, which supports initiatives that empower and assist women and girls in achieving self-sufficiency, two promising young scientists had the opportunity to join the CREW team for an internship that involved designing and conducting an independent research project in plant or animal conservation science.

CREW's Plant Lab has long worked with tissues from the endangered Florida Avon Park harebells (*Crotalaria avonensis*), but always pondered how much genetic diversity had been captured in the CREW collection. Therefore, 2018 Plant Lab Schmidlapp Scholar Diana Bolton was challenged with answering this question. Diana received her B.S. degree in Environmental Studies and Biological Sciences from the University of Cincinnati in December of 2017. Her diverse undergraduate research experiences included the development of markers for genetic analysis. She applied these skills to her work on the Avon Park harebells. In collaboration with Dr. Theresa Culley at UC, Diana analyzed the DNA of 144 plants sampled from three remnant wild populations. Although she identified some diversity, it appears all plants were derived from a single ancestral population that has since been separated into three fragments due to habitat loss. The next step is to determine how much of this limited diversity is captured in the samples stored at CREW.

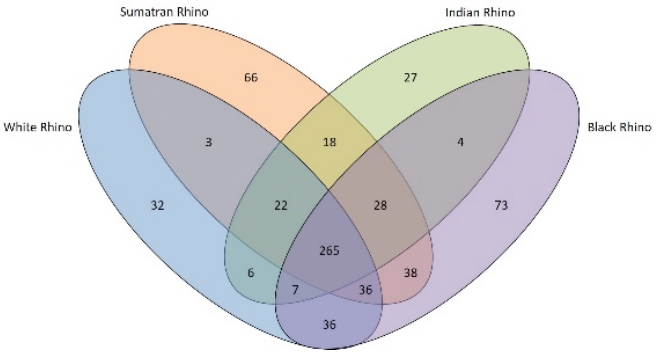


Kristen Counsell was selected from a competitive pool of candidates as the CREW Animal Division 2018 Charlotte Schmidlapp Scholar. Kristen received her B.S. degree from Idaho State University and her M.S. from Mississippi State University, where she worked under the guidance of CREW post-doctoral alumna Dr. Carrie Vance. Kristen's graduate research focused on profiling animal stress and reproductive states in the horse, giant panda, and Colorado boreal toad. After finishing her M.S., she performed fieldwork in both New Mexico and Puerto Rico, assessing amphibian populations and cryopreserving genetic samples. At CREW, Kristen will be identifying the primary glucocorticoid metabolite excreted in polar bear feces, which will enable scientists to more accurately assess stress/excitation in this species. Additionally, she will compare glucocorticoid concentrations in female polar bears that successfully produce cubs versus those that fail to reproduce to determine if stress could be a factor impacting reproduction.

Love Hurts, Love Scars, Love Wounds

Habitat loss and poaching have driven the wild population of Eastern black rhino (*Diceros bicornis michaeli*) to less than 1,000 individuals. With such few individuals remaining, maintaining a sustainable captive population is crucial for black rhino conservation efforts. The North American Eastern black rhino captive population includes 21 breeding pairs, but several of these pairs have yet to produce calves. CREW scientists are investigating the role of reproductive physiology and courtship behaviors in breeding success. Fecal samples and breeding behavior recorded via GoPro cameras have been collected from six rhino pairs housed at six different U.S. institutions. To date, three of the pairs have successfully bred – a shout out to our black rhino pair Seyia and Faru for conceiving Kendi! Preliminary data indicate no difference in estrous cycle length or progesterone and testosterone fecal metabolite concentrations between successful and unsuccessful breeding pairs. Interestingly, horn clash, jousting, advancing, and following behaviors are more commonly found in successful breeding pairs than in unsuccessful pairs. Hence, successful courtship and breeding in black rhinos involves aggressive behaviors. These behaviors may be associated with the release of glucocorticoids, i.e., "stress" hormones, and an imbalance in the output of glucocorticoids may suppress the expression of these behaviors. To find out, CREW scientists are in the process of measuring and comparing fecal glucocorticoid metabolite concentrations in relation to courtship behavior in successful and unsuccessful breeding pairs. (*This project is supported by a grant from the Institute of Museum and Library Services.*)

MicroRNAs: Mini Messengers Revealing Megafauna Mysteries



Shared and species-specific miRNAs in serum of four rhino species.

MicroRNAs (miRNA) are small molecules made up of nucleotides (the same building blocks of DNA and RNA). MicroRNAs respond to different physiological states such as illness, reproductive status and food digestion, in turn influencing gene-expression controlling biological processes within an organism. Since miRNAs respond to physiological changes that occur within the body, they can reveal a great deal of information about what an individual is experiencing. For instance, in cows, changes in miRNAs can be used to diagnose pregnancy as early as eight days post-conception, and in humans, miRNAs can be used to predict the development of Alzheimer's disease. Rhinoceros species are impacted by several unique diseases that are difficult to diagnose, and CREW scientists are interested in determining if miRNA concentrations in rhino serum may provide insight into the health status of individuals. Circulating miRNAs within the serum of 27 rhinos of 4 species (African black, African white, Indian, and Sumatran) were sequenced, revealing the existence of 661 different miRNAs within the rhinoceros taxon. Of those, 168 have not previously been reported and may be unique to rhino species. The next step of this study is to investigate how miRNAs differ between healthy animals and those afflicted by iron overload disorder, a disease that is difficult to diagnose. CREW scientists are hopeful that miRNA biomarkers may bridge the gap in our knowledge of how to diagnose the disease and allow for earlier treatment. (*Supported by a grant from The Eppley Foundation for Research.*)

OptiXcell: The Answer to Optimizing Rhino Sperm Cryopreservation?

Semen cryopreservation is an important assisted reproductive technology for maintaining genetic variation in managed populations of endangered species. To date, the semen extender (media used for freezing semen) of choice for most species contains egg-yolk or other types of animal protein. Unfortunately, egg-yolk runs the risk of microbial contamination and could result in the spread of disease when the semen is thawed and used in procedures such as artificial insemination. CREW has been on a quest to find a vegan alternative to egg-yolk that will reduce or eliminate this risk but also protect rhino sperm during freezing. It has not been an easy adventure, as it turns out soy-derived products (loved by cat and cow sperm) limit rhino sperm motility, and coconut products have an out-right murderous relationship with rhino sperm. Thankfully, we believe the hero we've been hoping for has been found! OptiXcell is a commercially available animal-protein free extender that protects bull and buffalo sperm during freezing. CREW tested OptiXcell with semen collected from three rhino species: African black, African white, and Indian, and preliminary results are promising. Sperm frozen in OptiXcell displayed post-thaw quality equivalent to, and in some cases better than, sperm frozen with the traditional egg yolk extenders. OptiXcell offers an option that can help to prevent possible disease transmission, but also protects invaluable genetic material while it is being stored, relieving the worries associated with the traditional egg yolk options. (*Study supported by a gift from the Coombe Family Fund of the Greater Cincinnati Foundation.*)

Collaboration is Key to Conserving Rhinos

This Fall marks the completion of a four-year National Leadership Grant from the Institute of Museum and Library Services to apply and enhance assisted reproductive technologies (ART) for captive African and Asian rhinos to ensure their optimal genetic management and long-term sustainability. This collaborative effort between CREW and SeaWorld Busch Gardens Reproductive Research Center (SWBGRRC) involved partnering with 28 Association of Zoos and Aquariums (AZA)-accredited institutions and three privately owned facilities to achieve project goals. One primary objective was to build upon national rhino gamete rescue centers by banking substantial sperm numbers and increasing the individual males represented. While sperm sexing technology has been established in African white rhinos, the grant facilitated banking additional x-enriched samples from this species and enabled successful development of this technology in two new species, the African black and Indian rhino. In total, 77 multi-thermal gradient tubes of x-sorted and non-sorted sperm, and over 1400 straws of non-sorted sperm were banked from 27 male rhinos representing three species. Additionally, the first use of a mobile laboratory (ST Genetics) to sort and freeze rhino sperm occurred over this past year. Previously, semen had to be hand carried on an airline flight from each zoo where it was collected to the SWBGRRC lab in San Diego for sorting, whereas the mobile lab could be reached within a 1.5 hour drive. In addition to minimizing the time and hence stress that sperm samples had to endure from collection to sorting, the mobile lab employed three next-generation sorting machines, which increased the sort rate from 3,000 to 8,000 sperm/second. Future AI procedures may allow us to produce rhinos of predetermined sex for improved population management.

Collaborating scientist from ST Genetics overseeing the sorting of X- and Y-enriched sperm from an African white rhino in the mobile laboratory.

The Missing Lynx: Characterization of Basal Seminal Traits and Sperm Cryopreservation in Canada Lynx

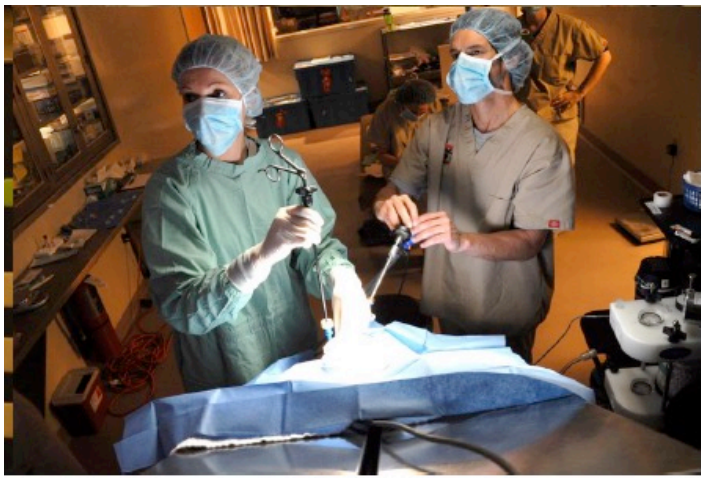
Being the missing lynx is really tufted! The Canada lynx (*Lynx canadensis*), with its striking tufted ears, has received little research attention in zoos so information about its reproduction is very limited. In North American zoos, this species is managed under a Species Survival Plan (SSP), but the current population is not sustainable. Moreover, Canada lynx have experienced reproductive boom and bust cycles, complicating genetic management of the population under human care. Increased knowledge of their reproductive biology would benefit both breeding management and development of assisted reproductive techniques. We recently initiated a project to describe basal seminal parameters in this elusive species and explore the feasibility of semen cryopreservation. To define the best window of sperm collection and assess the effect of seasonality on lynx seminal traits, semen and fecal samples (for testosterone measurement) were collected before (late January), during (mid-February to mid-March) and after (early April) the peak winter breeding season. During the 2018 breeding season, CREW scientists recovered sperm from all nine males via electroejaculation. Preliminary results showed that sperm parameters were similar among the collection periods. Overall, sperm production and semen quality in lynx were low in most males, but comparable to those reported previously in other lynx species. In addition, semen was frozen from eight of the lynx, using protocols that proved suitable in other felid species. Assessment of frozen sperm traits after thawing, and analysis of fecal testosterone levels are under way. Together, these results will provide zoos and population managers with a database of normal reproductive parameters in male lynx, and hopefully help to improve population sustainability. (*Supported by a grant from the Institute of Museum and Library Services and the Roger & Kathy Gross Postdoctoral Fellowship.)*

An Awful Lot of News about an Awesome Lot of Ocelots

AI kitten at Arizona Sonora Desert Museum

Of the many wild cat species studied at CREW, ocelots have received special emphasis for a variety of reasons. First, they are an American cat, native to the southwestern US, but now barely surviving along the Texas border with Mexico. Although the ocelot species is not endangered in Latin America, the US ocelot population is most definitely in dire straits, with fewer than 60 individuals currently existing in the wild. Second, ocelots have been a focal felid species at the Cincinnati Zoo over the past 40 years, with 44 kittens born in that time span, including the first ocelot produced from in vitro fertilization and embryo transfer, and one of the first ocelots born from artificial insemination (AI). Third, CREW has been responsible for developing the reproductive knowledge and expertise that is now helping to conserve the ~100 ocelots managed in zoos by the Ocelot Species Survival Plan, a program coordinated by CREW's Dr. Bill Swanson. Just in the past year, CREW scientists produced their seventh pregnancy in ocelots with AI, resulting in the birth of a healthy female kitten at the Arizona Sonora Desert Museum. CREW also initiated a semen cryopreservation project for wild ocelots in south Texas, working with Dr. Hilary Swarts, the USFWS biologist at the Laguna Atascosa National Wildlife Refuge and Dr. Tom DeMarr, the head veterinarian at the Gladys Porter Zoo. Using CREW's field-friendly methods for semen collection and ultra-rapid freezing, Drs. Swarts and DeMarr are banking semen from wild ocelots in conjunction with their ongoing health monitoring and radio-collaring efforts. This frozen semen will be stored in CREW's CryoBioBank until needed for future AI procedures with ocelots in zoos or possibly living in the wild. (*Supported by the Institute of Museum and Library Services.*)

Cat Doctors Without Borders



Dr. Lűders (left) and Dr. Swanson conducting laparoscopy

CREW's expertise in using assisted reproduction to propagate endangered cat species is well-known throughout the world. Our cat program has trained post-doctoral scientists originating from Australia, Brazil and Spain and aided collaborators in the United Arab Emirates and Brazil to produce sand cats and ocelots, respectively, via assisted reproductive technology. To help spread these reproductive skills to two new countries, CREW recently hosted two European zoo veterinarians – Dr. Imke Lűders from the Műnster Zoo (Germany) and Dr. Rui Bernardino from the Lisbon Zoo (Portugal) – for an intensive one-week training course. CREW scientists instructed their colleagues in the techniques of laparoscopy and laparoscopic oviductal artificial insemination (LO-AI), working with CREW's domestic cats. Both veterinarians became adept at conducting LO-AI after a short period of time, and even produced a domestic cat pregnancy following one of their practice AIs (using a semen sample frozen for ~20 years!). To our knowledge, this frozen semen is the oldest ever used to produce live offspring in the domestic cat and reinforces the value of semen freezing for long-term preservation of felid genetic potential. Now back in Europe, Drs. Lűders and Bernardino will be applying their new reproductive skills in an ongoing conservation project with the endangered Persian leopard, using LO-AI with frozen semen to create genetic exchange among leopards housed in different European zoos.

Unraveling the MIS-tery of Non-Surgical Sterilization for Free-Roaming Cats

Sterilization has long been recognized by animal welfare experts as a key strategy to reduce the number of cats entering and killed in shelters each year. Surgical sterilization (i.e., spaying and neutering) is expensive, labor-intensive, and requires a veterinarian; and thus cannot fully address this monumental problem. However, non-surgical approaches are now being developed that may one day augment traditional spay-neuter programs.

One promising new method involves a hormone termed MIS (Mullerian Inhibitory Substance) that is naturally produced by the ovaries to help regulate the number of eggs that develop each cycle. When present at high levels, MIS is a potent inhibitor of egg development and represents an attractive candidate for non-surgical sterilization. CREW recently completed a one-year pilot study to investigate a novel approach to producing MIS: recombinant adeno-associated virus (AAV)–delivered gene therapy. Our collaborators at Harvard University developed an AAV construct that encodes for the MIS protein, essentially borrowing the protein-making machinery of the cat's muscle cells to produce MIS. Three CREW cats were treated, with all producing high levels of MIS that suppressed ovarian activity for two months before cyclicity resumed. Our leading hypothesis for the temporary suppression is that the MIS protein was too immunogenic, provoking the cat's immune system to develop anti-MIS neutralizing antibodies. Our Harvard collaborators have created a new AAV construct that should resolve the problem and allow MIS levels to remain elevated much longer (potentially for the cat's entire life). CREW is currently gearing up for the next vectored contraception study, scheduled to begin in October. These groundbreaking studies represent a major milestone toward humanely reducing free-roaming cat populations and eliminating shelter euthanasia of healthy cats. (*Funded by the Joanie Bernard Foundation and Michelson Found Animals Foundation.*)

Sifting Through the Sandwort Genome

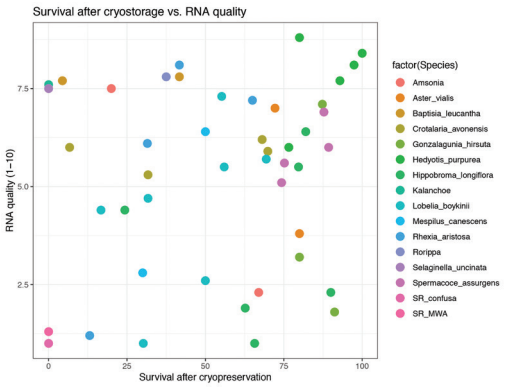
Cumberland sandwort (*Minuartia cumberlandensis*) is a federally endangered plant found on the Cumberland plateau of southern Kentucky and northern Tennessee. In 2005, to aid the conservation and management of the species, CREW developed a tissue culture propagation protocol and outplanted 63 tissue culture plants in the Daniel Boone National Forest. Since that outplanting, the population has grown to nearly quadruple its original size! Because tissue culture propagation is clonal, however, we wanted to understand how well our outplanting matches the natural populations in terms of genetic diversity. Developing microsatellite markers in the species proved tricky, as the species has low genetic diversity in general and we were unable to get enough data to distinguish individuals from one another. Using the unconventional sequence-related amplified polymorphism (SRAP) marker system, we were able to gain a better understanding of the population genetics of the species. As expected, the natural populations exhibited the highest genetic diversity. However, the diversity observed in our tissue culture outplanting was very close to that of the natural populations. In addition, while some evidence for clonal propagation in all of the populations was observed, there weren't significant differences in the genetic makeup of the populations. What can we take away from all this? Tissue culture propagation is a fantastic tool for the conservation of this species, and we already have a successful example of it in use!

Plants Under Fire: Pennyroyal Conservation

Todsen's pennyroyal (*Hedeoma todsenii*) is a federally endangered plant from New Mexico. This plant likes to grow up on the mountain slopes to escape the New Mexico heat, but it also has to contend with another obstacle: missiles! The majority of the species is found on the White Sands Missile Range, an active missile testing facility for the U.S. Army. Because the plant rarely produces viable seed, CREW has been working on cryopreserving the species for almost 20 years. Samples removed after 4-16 years of cryostorage were tested for their viability, and those stored across this time range did survive after cryopreservation. A new method of cryopreservation, droplet vitrification, was also tested in the species and improved survival rates to 72% from the 24-39% observed with other methods. To make certain our ex situ collection accurately captures the genetic diversity found in the species, we sampled the species' entire geographic range for genetic analysis. Low genetic diversity was found in the species, and even though there are many geographically distinct populations, we could distinguish only two major populations genetically. This information will be used to prioritize areas for our future *ex situ* conservation efforts. Moreover, our findings are useful to land managers striving to keep the species listed under the Endangered Species Act because we now know there aren't 30+ distinct populations, there are only two! (*Funded in part by grants from the Institute of Museum and Library Services and the U.S. Army.*)

How Stable are our Frozen Genes?

As part of our Institute of Museum and Library Services National Leadership Grant to evaluate the survival of seeds and tissues in long-term cryostorage, we tested the RNA quality of tissues after cryostorage. Previous studies in soybeans have suggested that length of time in conventional storage and seed viability can affect RNA quality. In our study, however, no relationship was found between the quality of RNA and either the length of time in storage or the survival after storage. There are a variety of possible reasons for this finding, but most importantly, it doesn't appear that reduced RNA quality should be a major concern in cryopreserved samples. In addition to RNA quality, we tested samples that had been stored in either tissue culture or cryopreservation to see if DNA remained stable during storage. Genetic changes were observed in both the tissue culture and cryopreserved samples, although cryopreserved samples had a lower rate of change. CREW interns are currently running a follow-up experiment to determine when these changes may occur and whether they can revert after the initial mutation. However, these results suggest that cryopreservation is preferable to tissue culture for promoting genetic stability in samples stored long-term.



SCIENTIFIC HIGHLIGHTS

BOOK CHAPTERS

WF Swanson and LM Penfold. 2018. Captive wildlife breeding: mammals & birds. In: Skinner M (ed.), Encyclopedia of Reproduction, 2nd Edition. New York, NY: Academic Press; Pp. 782-790.

PEER-REVIEWED PUBLICATIONS

Brennan AN, **VC Pence**, MD Taylor and M Westwood. 2017. *In vitro* growth responses of 12 North American oak species using two different media. HortTechnology 27(5), Oct. 2017.

Bryant JL and **TL Roth**. 2018. Annual fecal glucocorticoid metabolite concentrations in pregnant and pseudopregnant polar bears (*Ursus maritimus*) in North American zoos. Journal of Zoo and Aquarium Research 6(1):6-11. DOI:https://doi.org/10.19227/jzar.v6i1.259

Calatayud NE, **M Stoops** and BS Durrant. 2018. Ovarian control and monitoring in amphibians. Theriogenology 109:70-81. https://doi.org/10.1016/j.theriogenology.2017.12.005

Fischer A, VAW Benka, J Briggs, MA Driancourt, J Maki, DSO Mora, K Morris, KA Myers, L Rhodes, **LM Vansandt**, GR Weedon, J Wolf, and JK Levy. 2017. Effectiveness of GonaCon as an immunocontraceptive in colony-housed cats. Journal of Feline Medicine and Surgery 20(8):786-792.

Mays Jr. HL, Chih-Ming Hung, PJ Shaner, J Denvir, M Justice, Shang-Fang Yang, **TL Roth**, DA Oehler, Jun Fan, S Rekulapally and DA Primerano. 2018. Genomic analysis of demographic history and ecological niche modeling in the endangered Sumatran Rhinoceros *Dicerorhinus sumatrensis*. Current Biology 28(1):70-76. DOI: https://doi.org/10.1016/j.cub.2017.11.021

Metrione LC, **HL Bateman**, **WF Swanson** and LM Penfold. 2018. Characterization of the behavior and reproductive endocrinology of giant river otters (*Pteronura brasiliensis*) in managed care. Zoo Biology DOI:10.1002/zoo.21441

Pence VC, **M Philpott**, TM Culley, **B Plair, SR Yorke**, **K Lindsey**, **A-C Vanhove** and **D Ballesteros**. 2017. Survival and genetic stability of shoot tips of *Hedeoma todsenii* R.S. Irving after long-term cryostorage. In Vitro Cellular and Developmental Biology—Plant 53(6), October 2017.

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Roth TL and **WF Swanson**. 2018. From petri dishes to politics: a multi-pronged approach is essential for saving endangered species. Nature Communications 9:2588.

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Stoops MA, **GD Winget, CJ DeChant**, RL Ball and **TL Roth**. 2018. Early fetal sexing in the rhinoceros by detection of male-specific genes in maternal serum. Molecular Reproduction & Development 85(3):197-204. https://doi.org/10.1002/mrd.22953

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Wojtusik J, **MA Stoops** and **TL Roth**. 2018. Comparison of soy lecithin, coconut water, and coconut milk as substitutes for egg-yolk in semen cryodiluent for black rhinoceros (*Diceros bicornis*) and Indian rhinoceros (*Rhinoceros unicornis*). Theriogenology 121:72-77.

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Gonzalez R, J Newsom and **WF Swanson**. 2018. Ultra-rapid freezing of domestic cat spermatozoa for potential application to endangered felids. CRYO2018, The 55th Annual Meeting of the Society for Cryobiology, p. 3 (abstr. S9). Oral presentation, Madrid, Spain.

SCIENTIFIC HIGHLIGHTS

Hansel N and **VC Pence**. 2018. Survival of hyperhydric and more normal phenotypes of *Cycladenia humilis* var. *jonesii* and *Crotalaria avonensis* through cryopreservation. In Vitro Cellular and Developmental Biology–Plant 54:S56. Society for In Vitro Biology meeting, Poster presentation, St. Louis, MO.

Kennedy VC, **TL Roth**, **EM Donelan** and **E Curry**. 2018. Western blot evaluation of fecal transthyretin (TTR) for use as a pregnancy test in polar bears (*Ursus maritimus*); In search of the golden compass of population management tools. Proceedings of the Society for the Study of Reproduction, P447. Poster presentation, New Orleans, LA.

Lambo CA, KD Paterson , **WF Swanson** and SJ Jaques. 2018. Serum anti-müllerian hormone concentration during controlled ovarian stimulation in felids for improved monitoring of reproductive potential. Proceedings of the Joint Meeting of the American Association of Zoo Veterinarians, European Association of Zoo Veterinarians and Leibniz Institute for Zoo and Wildlife Research. Oral presentation, Prague, Czech Republic.

Pence VC. 2018. Challenges in the cryopreservation of endangered wild species. CRYO2018, The 55th Annual Meeting of the Society for Cryobiology. Invited speaker, Madrid, Spain.

Pence VC. 2018. Meeting the challenge of exceptional plants conservation: Conserving species without bankable seeds. Kew Science Seminar. Invited speaker, Royal Botanic Gardens, Kew, Richmond, UK.

Pence VC. 2018. Exceptional plant species conservation at CREW–USA. Workshop on exceptional plant species. Invited speaker, Royal Botanic Gardens, Kew-Wakehurst, Ardingly, UK.

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