Saving Species With Science®
CREW ReView
Lindner Center for Conservation and Research of Endangered Wildlife

Can you identify all CREW team members? Check next page for answers.

Dr. Terri L. Roth

VP of Conservation & Science and Director of CREW

ROTH'S REMARKS
CREW During COVID

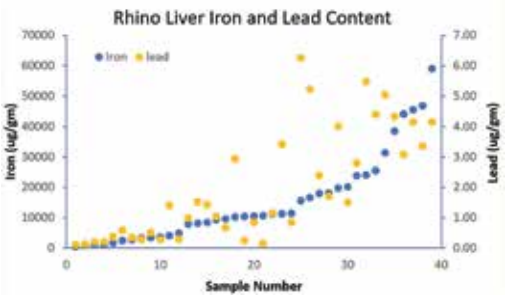
CREW was transformed in March of 2020 when the Governor shut down the state, and the Zoo closed its doors. Without the usual robust team of volunteers, interns, and students, CREW quickly went quiet, and then it went dark to save on utilities. Essential research continued with scientists working in shifts to ensure only one person per lab, while a few staff worked full weeks at CREW caring for the living collections of cats and plants, and even helping with animal care in the Zoo. Everyone adapted to change, working varied hours of the days, evenings and weekends to perform necessary work while remaining safe. CREW's typical, extensive travel ground to a halt, and instead, the senior staff worked from home writing long over-due papers and new grants, learning from free webinars, participating in virtual scientific conferences, giving virtual lectures, and responding to students across the nation interested in wildlife research but sitting home due to canceled internships. Updates were regularly provided to our many volunteers who were missing their CREW time. Weekly staff meetings continued virtually, and we soon learned just how hard it is to recognize each other when adorned with masks! As the state and Zoo slowly reopened, CREW staff spent a month on the Zoo's front-line scanning tickets, checking reservations, running the elevator, and parking cars. But, much as the sun brings the garden out of dormancy each spring, so too has the reopening slowly brought CREW back to life. Though still not its former bustling self, CREW has emerged from the quiet and darkness of late March with renewed energy and purpose as we carry on with our mission of Saving Species with Science (SAFELY!).

Key to cover

How did you do? Yes, we threw you a couple of curve balls with our new CREW Charlotte R. Schmidlapp Scholars, Claire and Kendra.

Rhinoceros *SIGNATURE* PROJECT UPDATES

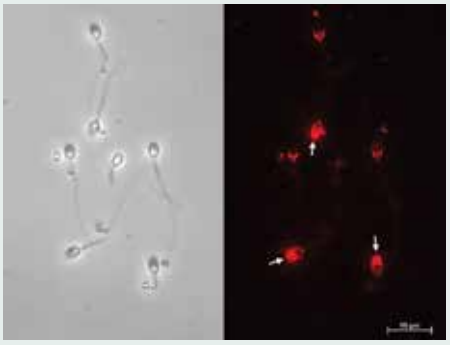
Mining for Answers to Iron Overload in Rhinos



As CREW's research on iron overload disorder (IOD) in rhinos has progressed, it has become clear that the etiology of the disease differs between black rhinos and Sumatran rhinos. For black rhinos, which rarely die from liver failure due to excessive iron stores, we have long questioned just how much IOD compromises their health and liver function. Answering that question requires direct liver tissue evaluation, which is simply not possible in living rhinos. The next best thing is to analyze liver tissue post-mortem, so that is exactly what has been happening over the past two years with the help and much-needed expertise of our colleagues at Michigan State University (MSU), St. Louis Zoo and Stellenbosch University. In 2019, Hailee Butler, an MSU veterinary student keenly interested in the field of pathology, was provided a stipend by CREW to work with expert MSU pathologist Dr. Dalen Agnew to prepare slides and tissues for analysis. To-date, 45 livers have been analyzed for mineral content, and now a subset of those are being evaluated for pathological changes that may reveal the impact on rhino health. However, the mineral data already are yielding some interesting results. For example, an unexpected positive correlation was found between iron and lead concentrations in the livers, suggesting black rhinos may be accumulating minerals more toxic than iron. Is this finding just incidental or is it an important piece of the puzzle? Time (and more research) will tell. (*This project was supported by a very generous anonymous CREW donor.)*

Painting the Rhino Sperm Red

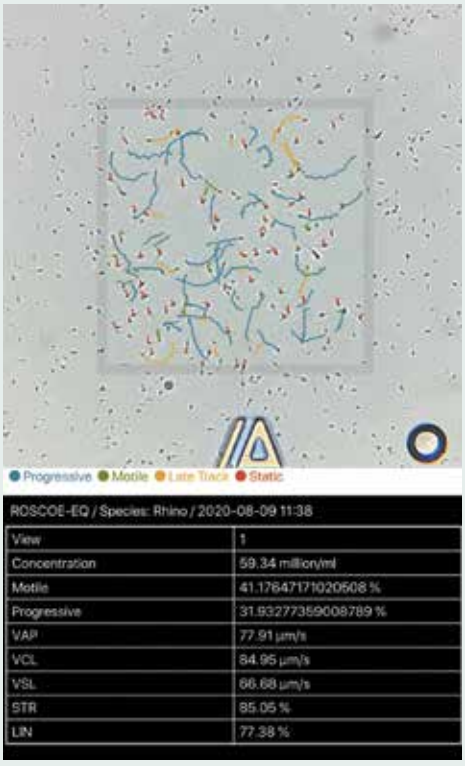
Sex ratio management is essential to building a sustainable and thriving population of rhinos. Preselecting the gender of offspring is possible by utilizing sex sorted sperm, i.e., sperm that has been separated into X-bearing (female producing) and Y-bearing (male producing) populations. The current technology for sorting has successfully been customized for rhino sperm by CREW's colleagues at SeaWorld San Diego. However, there are several drawbacks to the methodology, including high instrument cost (>$100K), the need for specialized expertise, long hours of labor, and the loss of up to 50% of sperm cells during the process. There may be a more user-friendly and cost-effective alternative that relies on targeting specific proteins (TLR7/8) on the sperm to reduce the swimming speed of the X-bearing sperm without affecting the mobility of the Y-bearing sperm. The first step in determining if this technology can be applied to rhinos involves evaluating sperm for the presence of the specific proteins. Antibodies labeled with a red fluorescent dye allow us to look for the presence of TLR7/8 on sperm cells through the microscope. This painting of sperm recently revealed that TLR7/8 are indeed on rhino sperm. Interestingly, ~50% of the sperm has staining on the top of their heads (white arrows), and ~50% do not. Now the question is, can this difference be used to separate the female producing sperm from the male producing sperm? *(Special thanks to Jackie Dieckman and Mike Camery for their gift that supported the fluorescent scope and camera upgrade to improve CREW's ability to see rhino sperm painted red.)*



Same slide shown under phase contrast (left) and fluorescence (right).

Spy with the iSperm

Before freezing semen to become part of the CryoBioBank® at CREW, it is essential to evaluate the swimming skills of sperm within the sample. Specifically, how many of the sperm are swimming (i.e., motile) and in a mostly straight path (i.e., progressively motile)? Sperm that swim slowly or in circles tend to be unsuccessful at fertilization, especially after the cryopreservation process. However, accurate assessment of sperm motility can be challenging for humans. When watching sperm on a microscope, we tend to focus on those swimming, and the non-motile sperm kind of fade into the background. And because each person sees things a little differently, motility values from the same sample often vary from person to person even after extensive training. Technology adopted by human IVF clinics and livestock industries overcomes this bias. Computer assisted sperm analysis (CASA) uses recorded images and software to accurately measure sperm movement, marking each sperm as non-motile or motile. With CASA, after less than a minute, we know not only how many sperm are motile, but also their swimming speed and the concentration of the sperm. CREW currently is beta testing rhino specific software for a portable sperm analyzer, the iSperm. Aidmics Biotechnology, the inventor of the microscope system that mounts on an iPad mini, has generously provided the setup free of charge to CREW. Once validated, iSperm for rhinos could be used to standardize evaluations across the many facilities with staff now trained to collect and bank rhino semen. It would remove the human bias and provide quality control assurance to those requesting samples for rhino ART procedures in the future.



Rhino sperm tracking by iSperm

Nationwide Network Tackles the Nuts and Bolts of Exceptional Species Work

What will it take to conserve exceptional species (i.e., rare plants not amenable to seed banking)? How do we get more institutions and researchers involved in this massive undertaking? Our current grant from the Institute of Museum and Library Services provided funding for research projects on exceptional species in 11 other institutions over the past year to help answer these questions. In September, CREW hosted a virtual meeting with these researchers who reported their results, discussed successes and challenges, and combined their experiences to evaluate what it will take to cryopreserve the endangered exceptional species of the world. The projects are wide-ranging and include developing *in vitro* propagation protocols for targeted endangered species (Missouri Botanical Garden, Huntington Botanic Garden, Chicago Botanic Garden, Lyon Arboretum), seed germination protocols and characterizing seed storage behavior for suspected short-lived and recalcitrant seeded species (Denver Botanic Garden, Atlanta Botanic Garden), germination and cryopreservation protocols for seeds of rare orchids (Fairchild Botanic Garden, San Diego Zoo Global, Longwood Gardens), and embryo cryopreservation protocols (National Laboratory for Genetic Resources Preservation). All of these approaches will be needed in the effort to conserve exceptional species. The group will also evaluate the time and costs that went into their work. This information will provide an invaluable reference for justifying needs for future funding, training, and resources in the effort to conserve all plants species.

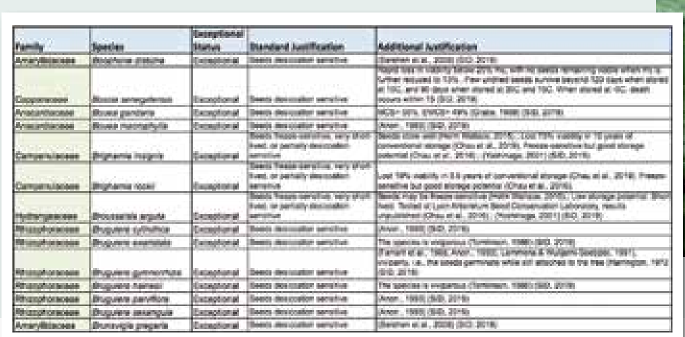
Researchers of the national exceptional plant network gathered at CREW in 2018 to present project ideas.

When Inconsistent Results may be Reason to Celebrate

Melicope mucronulata is the latest species we've finished banking in our current IMLS-funded project to cryopreserve Hawaii's endangered exceptional flora. This species is currently known from only three individuals in the wild, but the Lyon Arboretum in Honolulu maintains an *ex situ* population of about 21 genotypes in tissue culture. In the wild, this species is threatened by habitat loss and degradation by invasive feral goats. While we were banking all the genotypes in the *ex situ* population, we noticed that some individuals showed high survival after liquid nitrogen exposure using our established protocol, but others did not. To improve survival, we tested a different protocol developed by the USDA for use in citrus cryopreservation, because *Melicope* is in the citrus family. The response to this protocol was varied – some individuals survived much better on the CREW protocol, some on the USDA protocol, and some had a similar reaction to both. Stranger still, the response to the two different protocols was not correlated with how related two individuals were. Since the species is so critically endangered, there are only three mothers from which to collect seedlings. Seedlings from the same mother, which we would expect to be genetically similar, have completely different reactions to our two protocols. This gave us a hint that there may be more genetic diversity in the species than we expected from its small population size. To investigate this further, our new graduate student, Rachel Bridgens, is now undertaking a genetic study of the species to quantify diversity.

A Global Effort to Enumerate the Exceptional

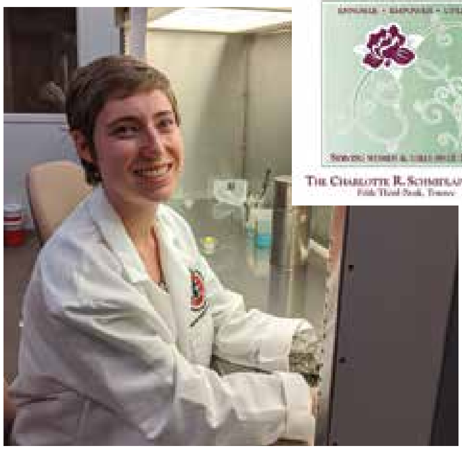
Although predictive models suggest that the number of exceptional plant species in the world may be in the tens of thousands, information on exactly which species are exceptional is critical to formulating and implementing strategies for dealing with their conservation. For the past several years, CREW's Plant Division has been working with Botanic Gardens Conservation International (BGCI) to develop and publish a Global List of Exceptional Species. That List will be available this fall. The process began with a list of North American Exceptional Species compiled by Longwood Gardens graduate student, Sara Helm Wallace, in collaboration with CREW and BGCI. Since then, a global list has been created based on seed storage behavior information from the Millennium Seed Bank's Seed Information Database, added to the North American List and a published Hawaiian species list. This was cross-referenced with the IUCN RedList and BGCI's Threat Search databases to determine which exceptional species were threatened. Lists of congeners of and suspected exceptional species and genera with exceptional species are also being compiled. These lists will form a major part of the Exceptional Plant Conservation Network Website, funded by our current grant from the Institute of Museum and Library Services. This first draft of about 1,000 exceptional species is well below the projected number, and the list is expected to grow over time with input from researchers around the world. However, it represents the first step to providing a basis for prioritizing research and identifying knowledge gaps, which should help advance the conservation of exceptional species.



Family	Species	Exceptional Status	Standard Justification	Additional Justification
Amaryllidaceae	Boophone disticha	Exceptional	Seeds desiccation sensitive	(Sershen et al., 2008) (SID, 2019)
Capparaceae	Boscia senegalensis	Exceptional	Seeds desiccation sensitive	[illegible]
Anacardiaceae	Bouea gandaria	Exceptional	Seeds desiccation sensitive	[illegible]
Anacardiaceae	Bouea macrophylla	Exceptional	Seeds desiccation sensitive	Anon., 1993 (SID, 2019)
Campanulaceae	Brighamia insignis	Exceptional	Seeds freeze-sensitive, very short-lived, or partially desiccation sensitive	[illegible]
Campanulaceae	Brighamia rockii	Exceptional	Seeds freeze-sensitive, very short-lived, or partially desiccation sensitive	[illegible]
Hydrangeaceae	Broussaisia arguta	Exceptional	Seeds freeze-sensitive, very short-lived, or partially desiccation sensitive	[illegible]
Rhizophoraceae	Bruguiera cylindrica	Exceptional	Seeds desiccation sensitive	Anon., 1993 (SID, 2019)
Rhizophoraceae	Bruguiera exaristata	Exceptional	Seeds desiccation sensitive	The species is viviparous (Tomlinson, 1986) (SID, 2019)
Rhizophoraceae	Bruguiera gymnorhiza	Exceptional	Seeds desiccation sensitive	[illegible]
Rhizophoraceae	Bruguiera hainesii	Exceptional	Seeds desiccation sensitive	The species is viviparous (Tomlinson, 1986) (SID, 2019)
Rhizophoraceae	Bruguiera parviflora	Exceptional	Seeds desiccation sensitive	Anon., 1993 (SID, 2019)
Rhizophoraceae	Bruguiera sexangula	Exceptional	Seeds desiccation sensitive	Anon., 1993 (SID, 2019)
Amaryllidaceae	Brunsvigia gregaria	Exceptional	Seeds desiccation sensitive	(Sershen et al., 2008) (SID, 2019)

Broussaisia arguta

Schmidlapp Scholar Hunting for Hormones of Hyperhydricity



CREW's Plant Division is excited to welcome Claire Thelen as its 2020 Charlotte R. Schmidlapp Scholar. Claire is our first Schmidlapp Scholar to work partially remotely due to the pandemic, but she is also spending some quality time in the CREW labs. Claire recently received her MS degree from Bowling Green State University, where her thesis topic was "Effects of Plant-plant Airborne Interactions on Performance of Neighboring Plants Using Wild Types and Genetically Modified Lines of *Arabidopsis thaliana*." Her previous work on plant hormone signaling will be a major asset to her Schmidlapp project on the effects of hyperhydricity on endogenous hormone levels in *Cycladenia humilis* var. *jonesii*. *Cycladenia* may be a familiar name to CREW fans, as the Plant Research Division has been studying this desert species for many years to understand the underlying reasons for hyperhydricity, a condition in tissue culture in which plants take up excess water and exhibit decreased chlorophyll production and stunted leaf growth. Claire will be working with the mass spectrometry facility at the University of Cincinnati to measure the hormone levels of hyperhydric and normal *Cycladenia* plants both before and after cryopreservation. This research may help us improve our cryopreservation and conservation efforts for the endangered *Cycladenia*, and may also be applicable to other species affected by hyperhydricity.

Big Cats Get the Small Cat Touch – with Help from CREW and IMLS

Jaguars, tigers, snow leopards, and Amur leopards are four iconic big cat species managed within North American zoos. Due to poor breeding success, low founder numbers, and small population sizes, long-term sustainability of the zoo populations cannot be achieved with natural breeding alone. Thanks to a three-year grant from the Institute of Museum and Library Services (IMLS), CREW is now uniquely poised to address these challenges and help ensure the long-term viability of these large cat populations. Team Cat recently received a prestigious National Leadership Grant which, "supports projects that address critical needs of the museum field and that have the potential to advance practice in the profession so that museums can strengthen services for the American public." The project, entitled *Improving Assisted Reproduction in Imperiled Big Cats*, will build on CREW's prior success with small cats to develop and improve assisted reproductive technologies (ART) in the four priority big cat species. Specifically, CREW will significantly advance the application of semen banking and artificial insemination (AI) for the management of big cats in US zoos by (1) creating a functional genome resource bank (sperm bank) to serve as safeguard against the loss of genetic diversity and to provide frozen sperm for AI; (2) developing an efficient method for AI; and, (3) increasing the scientific capacity of the zoological community through advanced training in ART. CREW's Dr. Lindsey Vansandt will serve as the Project Director and will be working in partnership with Dr. Jason Herrick from Omaha's Henry Doorly Zoo and Aquarium. Successful completion of this project will greatly enhance the genetic viability and sustainability of big cat collections maintained in North American zoos.

INSTITUTE of Museum and Library SERVICES

Trolling for Bladder Cancer Genes in Fishing Cats

With recent grant funding from the Basis Foundation, CREW is continuing its research investigating bladder cancer (transitional cell carcinoma, TCC) in fishing cats. In collaboration with Dr. Leslie Lyons, a geneticist at the University of Missouri's College of Veterinary Medicine, we are exploring the underlying genetic basis for the alarming predilection of bladder cancer in this endangered Southeast Asian cat species. Historically, one-third of fishing cats that pass away in zoos show evidence of this disease that is rarely observed in other wild cat species. We suspect that fishing cats are prone to developing TCC, in part, because of interactions between their nature (i.e., an evolutionary history of eating fish in the wild) and nurture (i.e., a contemporary history of eating beef in zoos). Our past research suggested that their likelihood of developing TCC might be decreased by switching cats to a primarily fish-based diet. In our current study, we are seeking to gain a clearer understanding of the genetic predisposition that make fishing cats more susceptible to bladder cancer on both a species and individual basis. By comparing whole genome sequences of fishing cats with TCC to those without (and to domestic cats and other wild cat species), we can determine if any candidate genes exist that may increase TCC susceptibility. Screening fishing cats for those specific 'cancer' genes may allow us to identify individuals that require closer disease monitoring and possibly reduce the prevalence of deleterious TCC-linked alleles through more selective reproductive management.

Feline Friendly: The Positive Effects of Operant Conditioning

Animal shelters can be a scary place for a cat. Their stress can manifest in a variety of behavioral issues and health problems, reducing their adoptability, extending their stay in the shelter, and further exacerbating stress-related issues. Operant conditioning using positive reinforcement-based training methods such as clicker training has long been recognized in zoos as a tool to improve animal welfare through mental enrichment, improved animal confidence, and strengthened human-animal bonding. In a CREW study, Dr. Ana Basto, a Joanie Bernard Foundation-supported veterinary scholar, investigated the impact of clicker training on domestic cats for mitigating the harmful effects of stress and improving friendly behavior. Twelve CREW cats were clicker trained over a six-week period; six were trained for friendly behaviors (e.g., approaching humans, face rubbing, being held) and six were trained for practical skills (e.g., nail trim, medical exam). Twelve additional cats that were not clicker trained served as controls. A Stranger Approach Test (SAT) was performed before and after the training period to measure the cats' comfort level with humans, as a proxy for their, "adoptability." Seventy five percent of the trained cats improved their SAT scores after training. Additionally, the cats trained for friendly behaviors were 28 times more likely to show an improvement in their SAT score versus the non-trained cats. These results suggest that clicker training may be a simple and rapid way to improve the welfare and adoptability of shelter cats. *(This study was funded by the Joanie Bernard Foundation.)*

Dr. Ana Basto and feline friends

New Schmidlapp Scholar Working with Cats of a Different Sort

Kendra Esparza-Harris

CREW's Charlotte R. Schmidlapp Scholarship program provides young female scientists interested in wildlife conservation with the unique opportunity to receive hands-on research training during a five-month course of study at CREW. Although delayed a few months by the COVID pandemic, CREW welcomed new Schmidlapp Scholar, Kendra Esparza-Harris, to Team Cat in June 2020. Kendra's educational background includes a BS in Animal Science from North Carolina A&T State University and an MS in Animal Science from the University of Illinois. Most recently, she spent the past 1½ years in Senegal serving in the Peace Corps. With mentoring by Drs. Bill Swanson and Louisa Rispoli at CREW, Kendra is investigating a novel approach to sorting domestic cat sperm by sex (X vs Y) as a model for endangered felids. For this study, Kendra is assessing cat sperm for the presence of surface proteins (called Toll-Like Receptors, or TLRs) previously described for mouse sperm. In mice, activation of TLRs that are located only on X-chromosome bearing sperm reduces their motility, allowing X and Y sperm to be separated easily using a swim-up processing technique. Kendra's preliminary results using Western blotting and immunofluorescence have shown that cat sperm have the same TLRs as mice but with a differing distribution on sperm midpieces and tails. If these cat TLRs exert the same sex-specific function when activated, then routine separation of X and Y-bearing cat sperm may be feasible. By incorporating this sperm sorting technique into our AI procedures with wild felids, we may be able to preselect offspring gender for improved management of our felid populations.

Little One Donates Blood for Wild Bears

Most of you probably didn't know that male polar bears are called boars, but did you know that Cincinnati Zoo's resident boar, Little One, is helping with conservation research of wild bears? With climate change and human disturbances impacting the Arctic ecosystem, scientists are interested in how the immune systems of wild polar bears are responding to a changing environment. Recently, CREW scientists partnered with field researchers who wanted to determine how body condition, land use, and exposure to environmental pollutants are affecting specific immune markers in this apex predator. These markers had never been examined in this species and fresh blood was needed to validate the laboratory tests. So, CREW and the Cincinnati Zoo's veterinary staff collected several tubes of blood from Little One at his annual examination and shipped them overnight to the scientists. A commercially available canine test kit was used to successfully validate 5 (of 12) immune markers of interest using Little One's blood. Then, the researchers analyzed blood samples collected from wild polar bears from the Beaufort Sea population. Results indicated that bears with higher levels of circulating environmental pollutants, specifically polychlorinated biphenyls (PCBs), had lower immune markers, indicating that PCBs may suppress the immune system in this species. As polar bear land use, food sources, and exposure to environmental pollutants shift with a changing environment, it's essential to have tools available to assess immune function in this species. We're proud that the Cincinnati Zoo could contribute to this important research. There's so much we can learn from zoo bears that will support conservation efforts of their wild cousins.

Photo by: Duke Ruggles

CREW Collaborates with Canuck to Learn More Aboot Boars, Eh?

Dylan McCart

In 2016, CREW teamed up with Dylan McCart, Conservation Coordinator of the Canadian Polar Bear Habitat (CPBH) in Cochrane, Ontario to study polar bear fecal hormones. The CPBH is the largest facility in the world dedicated to the conservation and research of this species. It is also one of only two facilities that currently houses sexually mature males together in groups, a feat rarely accomplished in carnivores that fight fiercely for breeding rights in the wild. Now, as part of his graduate research at York University, Dylan is hoping to determine if adult males housed with other males exhibit comparable behaviors and fecal testosterone concentrations as solo males or males living with females. To achieve his goals, Dylan coordinated the collection of fecal samples from adult males housed in different living arrangements throughout the U.S. and Canada. Because CREW validated methodologies to quantify testosterone in polar bear fecal samples and has an extensive database for comparison, Dylan made the 925-mile trek from CPBH to Cincinnati in January 2020 to receive training in enzyme immunoassay techniques to quantify testosterone concentrations in his samples. He's currently planning his second trip to CREW to finish analyzing the remaining samples (and to stock up on Skyline chili). Identifying variables that influence hormone production and aggression in male bears will help us better understand the endocrine system of this species and will be useful in determining the optimal housing conditions of polar bears in human care. *(This work was supported by The International Association for Bear Research and Management Experience and Exchange Grant.)*

It Takes Two (to Three) Years to Tango

Polar bears in zoos play an important role in promoting conservation of their wild counterparts and provide scientists with a view into their physiology and how environmental changes may impact survival. Traditionally, polar bears were believed to reach sexual maturity at approximately five to six years of age and that is when polar bears in human care are recommended for breeding. However, recent studies of wild bears have shown that males as young as two years old may sire offspring, which is surprising because generally, the older, larger males will win competitions for breeding opportunities. To address this discrepancy in breeding age assumptions, CREW scientists assessed reproductive hormones in over 7,000 fecal samples collected from 30 polar bears from zoos across North America to determine when bears typically reach sexual maturity while not subject to competition for mates. Statistical analysis is underway, and preliminary results are compelling. It indeed appears that male polar bears reach sexual maturity at about two to three years of age and that their testosterone levels are impacted by season, age, and whether they are reared by their mothers or hand reared. As was expected, testosterone levels were greater during the breeding season (~Jan – May) when sperm production is at its peak in adults and increased with age (from 1 to 6 years of age) likely due to continued testicular development. It appears that male polar bears can be paired for mating much earlier than previously thought which could provide a much-needed boost to cub production in zoos. *(This project was made possible in part by the Institute of Museum and Library Services grant #MA-30-18-0461-18.)*

CREW WISHLIST

ANTIBODIES

Needed to pre-treat plates for our endocrine assays. One antibody aliquot is enough for 170 plates (or ~6,000 samples)! Cost: **$335.**

WINTERIZING MATERIALS FOR HOOP HOUSE

Items for providing "burrito" insulation for the second hoop house for overwintering plants behind CREW. Cost to complete winterization: **$185.**

CAT EXERCISE WHEEL

As you read in this issue of the CREW ReView, CREW scientists are studying operant conditioning training as a way to improve cat welfare. In hopes of both enriching the lives and improving the health of some of CREW's feline friends, an exercise wheel is requested: https://onefastcat.com/collections/shop-all/products/cat-exercise-wheel. Cost: **$199** (on sale!).

REPEAT PIPETTORS

These are absolutely essential for accuracy when running enzyme immunoassays, but they don't last forever. Cost: **$352** each (could use up to three).

DIY LAB MAINTENANCE TOOLS & TOOL BOX

CREW staff do as much maintenance on their equipment as they can with the small set of tools maintained in the CREW lab. A few additional wrench sets and a new organizer tool box are desired to keep us in business. Cost: **$120.**

POLAR BEAR BLOOD TESTS

CREW scientists want to investigate novel hormones in blood samples that may be useful for reproductive monitoring. Cost: **$400-$1300** (each kit's price varies).

GAMETE LAB PUMP

Needed for filtering our culture medium and assay buffers to ensure sterility. Cost: **$619.**

Thanks to the following for supporting CREW in Fiscal Year 2020

(April 1, 2019—March 31, 2020)

$100,000+

Mr. and Mrs. Roger W. Gross
Institute of Museum & Library Services

$20,000–$99,999

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SCIENTIFIC HIGHLIGHTS

PEER-REVIEWED PUBLICATIONS

Ballesteros D and **VC Pence**. 2019. Survival and growth of embryo axes of temperate trees after two decades of cryo-storage. CryoLetters 88:110-113.

Bourque J, JPW Desforges, M Levin, TC Atwood, C Sonne, R Dietz, TH Jensen, **E Curry** and MA McKinney. 2020. Climate-associated drivers of plasma cytokines and contaminant concentrations in Beaufort Sea polar bears. Science of the Total Environment 745, DOI:https://doi:10.1016/j.scitotenv.2020.140978

Buckley RM, RA Grahn, B Gandolfi, **JR Herrick**, MD Kittleson, **HL Bateman**, **J Newsom**, **WF Swanson**, DJ Prieur and LA Lyons. 2020. Assisted reproduction mediated resurrection of a feline model for Chediak-Higashi syndrome caused by a large duplication in *LYST*. Scientific Reports 10:64.

Curry E, JS Easley, **J Wojtusik** and **TL Roth**. 2020. Identification of mink (*Neovison vison*) fecal proteins during embryonic diapause and placental pregnancy for non-invasive pregnancy diagnosis in wildlife. Bioscientifica Proceedings 10:101-112.

Pence VC, **LR Finke** and RP Niedz. 2020. Evaluating a DOE screen to reduce hyperhydricity in the threatened plant, *Cycladenia humilis* var. *jonesii*. In Vitro Cellular and Developmental Biology–Plant 56:215-229.

Pollock KE, JK O'Brien, **TL Roth**, J Proudfoot, J Niederlander, L Micheas, TR Robeck and **MA Stoops**. 2020. Anti-Müllerian hormone in managed African and Asian rhino species. General and Comparative Endocrinology 294, DOI: https://doi.org/10.1016/j.ygcen.2020.113487

Roth TL, A Switzer, M Watanabe-Chailland, EM Bik, DA Relman, LE Romick-Rosendale and NJ Ollberding. 2019. Reduced gut microbiome diversity and metabolome differences in rhinoceros species at risk for iron overload disorder. Frontiers in Microbiology, DOI: https://doi.org/10.3389/fmicb.2019.02291

Walters C and **VC Pence**. 2020. The unique role of seed-banking and cryobiotechnologies in plant conservation. Plants People Planet, DOI: https:doi.org/10.1002/ppp3.10121

Wojtusik J, IMC Brandicourt, W Rice and **TL Roth**. 2020. Reproductive cycle and pregnancy monitoring in the common hippopotamus (*Hippopotamus amphibius*) through salivary analyses and transabdominal ultrasonography. Journal of Zoo and Aquarium Research 8:181-187.

SCIENTIFIC PRESENTATIONS

Butler H, **T Roth**, D Agnew, M Duncan and J Buchweitz. 2020. A36 Black Rhino. Pathology Workshop Case Report. Proceedings of the Virtual Annual Meeting of the American Association of Zoo Veterinarians. Oral presentation.

Miller A and **VC Pence**. 2020. Factors affecting oak (*Quercus*) species rooting and cryopreservation protocols. Botany 2020 Virtual Botany Conference. Oral poster presentation.

Pence VC. 2019. Expanding *ex situ* conservation to all plants–The challenge of exceptional species. World Forum on Global Strategy for Plant Conservation (2019 GSPC). Invited oral presentation, Dujiangyan, Sichuan Province, China.

Pence VC. 2020. Temperature effects on recovery and growth of shoot tips of *Quercus virginiana* after liquid nitrogen exposure. Virtual 2020 World Congress on In Vitro Biology. Oral presentation. In Vitro Cellular and Developmental Biology 56:Suppl.

Pence VC. 2020. The effects of species and recovery temperature on the survival and growth of oak *(Quercus* spp.) shoot tips after cryopreservation. CRYO 2020 Virtual Annual Meeting. Invited oral presentation.

Philpott M, **AC Vanhove**, **S Yorke** and **V Pence**. 2020. Collecting exceptional species for *ex situ* conservation: Unique issues associated with tissue cryopreservation. Botany 2020 Virtual Botany Conference. Oral presentation.

Philpott M and **V Pence**. 2020. Differential survival of genotypes following cryopreservation in the endangered Hawaiian plant *Melicope mucronulata*. CRYO 2020 Virtual Annual Meeting. Oral presentation.

GRANTS AWARDED

Funding Source: The Institute of Museum and Library Services. Project: Improving Assisted Reproduction in Imperiled Big Cats. Role: Principal Investigator. Duration: 9/1/20 – 8/31/23. Amount: **$770,601.**

Funding Source: Basis Foundation. Project: Assessing the genetic basis and early diagnosis of transitional cell carcinoma in fishing cats. Role: Co-Principal Investigator. Duration: 06/20-05/21. Amount: **$6,000.**

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