CREW Puts its **Signature** on Wildlife Conservation

**Roth’s Remarks**

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

A dozen years ago, CREW’s senior staff worked with the Zoo’s Conservation Committee on developing a strategic plan. From that process emerged the idea of focusing the majority of CREW’s resources on a few carefully selected **Signature Conservation Projects** in an effort to make a meaningful difference for a few of the world’s imperiled wildlife species. This strategy is in contrast to that used by some other conservation organizations and zoos that seek to maximize the number of different species and countries with which they work. There is no right or wrong conservation strategy as long as zoos commit to one that contributes to saving the world’s biodiversity, but our decision to take a more focused approach has been extremely productive. For many years, CREW has worked primarily on three **Signature Conservation Projects:** 1) rhinos; 2) exceptional plants; and 3) small cats. This year, polar bears were added as our fourth **Signature** project. Over time, each program has experienced highs and lows and our scientists, in turn, have reveled in glorious successes and endured heart-wrenching disappointments, but one aspect has remained constant - we continue to march forward, and the results speak for themselves: 1) three Sumatran rhino calves born at the Cincinnati Zoo and a grandson recently born in Indonesia, three term Indian rhino pregnancies produced by artificial insemination (AI) with cryopreserved sperm and a fourth on its way; 2) hundreds of Avon Park harebells, Autumn buttercups and Cumberland sandworts propagated in test tubes at CREW and outplanted into the wilds of Florida, Utah and Kentucky, respectively; 3) ten litters of ocelots, Pallas’ cats and sand cats produced by AI or embryo transfer at four zoos in three countries; and 4) the first ever sperm banking and AI in polar bears. Although we continually feel the pressure to accomplish more and move faster, by staying focused and persevering, we are achieving our mission of making a significant impact on conserving a few of the world’s most endangered species.
Want to hear about Indian rhinos? Well, urine luck

Urineary hormones are used to monitor Indian rhino estrous cycles to time natural breeding or artificial insemination (AI). Just like us, rhinos can vary in hydration status. To control for variation in the water content of urine samples, hormone concentrations are indexed to creatinine. Creatinine, a by-product of muscle metabolism, is excreted into urine at a constant rate. Cincinnati Zoo rhino keeper staff routinely collect urine samples from our female Indian rhinos. These samples are stored frozen until they are thawed and analyzed in CREW’s endocrine lab. However, when an Indian rhino lives at another facility, urine is shipped to us and may have already been through several freeze-thaw cycles before it reaches our lab. Steroid hormones are very robust under extreme temperature conditions. However, creatinine is sensitive to degradation as a result of the freeze-thaw process. Another technique, urine concentration standardization by specific gravity is available, but had not been validated for Indian rhinos. Therefore, Shraddha Cantara, a 2013 P&G Wildlife Conservation Scholar at CREW, investigated the efficacy of specific gravity as an alternative for normalizing hormone concentrations in Indian rhinoceros urine. Shraddha examined urine samples collected over three estrous cycles in three female Indian rhinos to determine the robustness of both measures over the course of three freeze-thaw cycles. Shraddha’s study will help to improve the accuracy of hormone measurements for Indian rhinos no matter where they may reside.

Can High Cholesterol Actually be Good?

In humans, high cholesterol levels are known to be detrimental to our health, contributing to conditions such as arteriosclerosis, stroke and heart disease. However, cholesterol also is essential for a variety of bodily functions, including steroid production and cell membrane integrity. The challenge is to balance cholesterol concentrations with your body’s physical needs. A sperm cell faces the same dilemma in microcosm. The plasma membrane that surrounds a sperm cell contains cholesterol that helps to provide fluidity and maintain osmotic balance within the cell. When sperm cells are frozen, cholesterol is one factor that prevents membranes from easily fracturing. So if a little cholesterol is good, more is better – right? Not necessarily. In a study conducted at CREW, P&G Wildlife Scholar Isabel Plourde collected semen from domestic cats and added a little extra cholesterol to the sperm membranes, using cyclodextrins (water soluble sugar molecules) as the loading agent. Following freezing and thawing, sperm were evaluated for membrane integrity, motility over time and in vitro fertilization of domestic cat oocytes. With the highest cholesterol concentrations, very few sperm survived cryopreservation. With moderate levels of cholesterol, post-thaw sperm survival was acceptable, but fertilization success in vitro was lower than that for frozen-thawed control semen. So, at least in the domestic cat, extra cholesterol does not appear to be beneficial. However, Isabel’s results also showed that domestic cat sperm have higher basal cholesterol levels than sperm from two wild felids, the cheetah and fishing cat, suggesting that a slight increase in cholesterol just might help improve sperm cryopreservation in these endangered cat species.

P&G Wildlife Conservation Scholarship Program

With sponsorship from Procter & Gamble Pet Care, the Wildlife Conservation Scholarship Program was initiated in 2011 to give hands-on scientific training to veterinary students interested in pursuing wildlife conservation research as a career. In this collaborative partnership between CREW and the Ohio State University’s College of Veterinary Medicine, the P&G scholarship provides financial support to two OSU veterinary students each year to conduct wildlife research studies with co-mentoring by OSU and CREW scientists.

Predicting When Otters Oughta Give Birth

CREW’s non-invasive fecal hormone research in otters previously indicated that a sharp rise in progesterone concentrations associated with pregnancy and/or pseudopregnancy in both North American river (NARO) and Asian small-clawed (ASCO) otters could potentially be useful for predicting parturition dates. Specifically, the increase in progesterone occurs coincident with embryo implantation in the NARO which experiences delayed implantation and with ovulation in the ASCO which does not undergo embryonic diapause; however, it should be noted that pregnancy cannot be distinguished from pseudopregnancy. Nevertheless, since 2008, CREW scientists have been predicting the parturition window based on the progesterone increase in potentially pregnant NARO and ASCO, maintained at 25 U.S. Zoos. This year, overall results for 26 NARO and 9 ASCO females were presented at the Wild Musteloid Conference at Oxford University, UK. So, how did we do? In NARO, 30 distinct luteal phases were detected and 14 (47%) were subsequently confirmed as pregnancies, whereas the remainder were presumably pseudopregnancies (no pups observed). In ASCO, 31 luteal phases were identified, comprising 8 (25%) proven pregnancies and 23 presumed pseudopregnancies. In NARO, 10 (41%) females gave birth within the 63-75 day parturition window following the progesterone increase and in ASCO, 6 (87%) gave birth within the 68-76 day predicted parturition window. What does this mean? We can now fairly accurately predict the 1-1 ½ week interval during which pups can be expected from pregnant otters of both species. This information is greatly appreciated by animal care takers who want to be sure their husbandry practices optimize chances for successful reproduction.

World Premiere of THE BLUE AND GOLD

In October, 2013, the College of Mount St. Joseph in collaboration with the Cincinnati Zoo hosted the debut of a documentary that highlights a project led by Mt. St. Joseph’s alum, Bernadette Plair, during her career working at CREW. The goal of the project was to reintroduce blue and gold macaws to the island of Trinidad where they had lived forty years earlier, prior to being extirpated for the pet trade. The project was initiated in 1993, and has resulted in a flock of 86 blue and gold macaws now freely flying over the swamps of Trinidad. The film touches on all aspects of the reintroduction, from the research involved to the release of the pioneer flock, and includes the essential support from the Trinidad government and international organizations. Perhaps most importantly, the documentary highlights the involvement of the community bordering the Nariva Swamp that played a key role as “citizen scientists” to help document the macaw nesting sites and protect the birds in the wild. Due to its tremendous success, the reintroduction of blue and gold macaws to Trinidad is now serving as a template for other parrot reintroduction projects. The documentary has been selected for screening at the 8th Annual Trinidad and Tobago Film Festival and is nominated for several awards.

CREW’s P&G Wildlife Scholar Isabel Plourde

CREW’s P&G Wildlife Scholar Shraddha Cantara

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**Sniffing out a Pregnancy Test for Polar Bears**

Traditional methods of pregnancy detection, such as progesterone monitoring and ultrasound examination, are ineffective and/or logistically challenging for diagnosing pregnancy in the polar bear, so scientists at CREW have gotten creative. In collaboration with professional dog trainer Matt Skogen at IronHeart High Performance Working Dogs and a beagle named “Elvis”, they are trying to determine if the sensitive noses of canines can distinguish a pregnant polar bear from a non-pregnant bear by the smell of its fecal samples.

There is growing interest in the utilization of dogs for medical purposes. Trained sniffing dogs are employed in a variety of roles, including the detection of food allergens, the onset of seizures in epileptics, and low blood sugar in diabetics. Some groups have even trained dogs to distinguish breath, blood, and fecal samples originating from human patients with lung, ovarian, and colon cancers from those of healthy individuals.

For CREW’s study, over 200 fecal samples from polar bears of known pregnancy status were chosen retrospectively and sent to IronHeart, where Elvis is learning to identify the samples that came from pregnant females. He is currently working at a high accuracy level, but his real test will occur later this autumn, when he will be presented with samples from female polar bears around the country that might be pregnant. Determining the component of the feces that Elvis recognizes in the pregnant samples may allow researchers to work backwards and finally identify a polar bear pregnancy factor.

**Fertility Treatments for Barren Bears**

Due to growing concern over reproductive failure in polar bears nationwide, CREW scientists are beginning to receive requests from other zoos to perform assisted reproductive procedures, such as artificial insemination (AI). However, unlike human infertility treatments that have been thoroughly investigated and tested over the years, appropriate treatments for polar bears are completely unknown. In fact, until CREW scientists became involved, no one had even attempted to control the reproductive cycle of a polar bear to prepare it for AI. Such efforts are needed to synchronize ovulation with timing of AI to maximize the odds of sperm and egg meeting in the oviduct for fertilization while both gametes are still viable. After some very promising results following the first attempt to stimulate ovarian activity and induced ovulation in conjunction with their first AI attempt in 2012, CREW scientists decided to evaluate the effectiveness of the hormone regime more systematically this year. During the 2013 breeding season, three female bears, including the Cincinnati Zoo’s female, Berit, were given two-to-four hormone injections in an attempt to stimulate follicular development and ovulation. In polar bears, it is difficult to perform ultrasounds to visualize ovarian structures or to draw blood samples for hormone analysis, so responses are monitored by non-invasive methods. Prior to and following hormone injections, behavioral observations were conducted to determine if the females became receptive to their male partners and likely ovulated. Additionally, fecal steroid metabolite hormones are being analyzed to characterize each female’s response to the hormone regimen and to monitor for potential pregnancy or pseudopregnancy post-treatment. Preliminary results suggest that ovarian activity and ovulation can be induced with hormone injections in some females but that other females are non-responsive. Understanding how to control ovarian activity will lead to improved chances of success in future AI endeavors and may even assist with natural mating.

(CREW’s Polar Bear Signature Project is supported by a challenge grant from the Young Family Foundation and the Reuben Herzfeld Family Fund of the Greater Cincinnati Foundation.)

**Diving into the Fountain of Youth: Seeds return to life after decades in liquid nitrogen**

Is liquid nitrogen safe enough for long-term storage of plants? In order to answer this question with empirical data, several hundred samples stored in the Frozen Garden of CREW’s CryoBioBank will be removed, grown, and analyzed during the next three years. Thanks to this unique project at CREW, funded by the Institute of Museum and Library Services (IMLS), this will be the first time that viability and genetic integrity after long-term storage in liquid nitrogen will be studied for such a wide variety of samples, such as seeds, shoot tips, recalcitrant embryos, spores, and gametophytes. Some of the samples have been stored for more than 20 years, which provides one of the oldest and most diverse collections of plant germplasm in the world.

The first samples that have been removed from liquid nitrogen are orthodox seeds. Orthodox seeds are dried during maturation in the plant and can be easily frozen in seed banks. Germination is the simplest way to analyze seed viability, and it produces seedlings that develop into adult plants. Some of the seeds stored belong to endangered species and the resulting plants can be used to augment or restore populations, when and where they are needed.

Initial data suggest that seeds from poplars and willows, which are very short-lived and die at ambient conditions in just 2 or 3 weeks, have preserved their initial viability and vigor for more than 15 years! So, is liquid nitrogen the Fountain of Youth? So far the data look promising, but the project has just begun, and many more samples need to be tested.

**Build Me Up Buttercup—Don’t Break My Heart**

In June, 350 plants of the Autumn buttercup (Ranunculus acris) propagated at CREW were planted in the preserve that protects this species in the small region of Utah where these plants are known to exist. In the early 2000s, CREW’s Plant Division developed a protocol for propagating the plant through tissue culture, thanks to funding from the Institute of Museum and Library Services. Once this protocol was established, CREW became part of a team that included The Nature Conservancy, U.S. Fish and Wildlife, The Arboretum at Flagstaff, and Weber State University, all working together to restore the buttercup in its native habitat. June’s outplanting was the third and largest attempt. The planting in 2007 resulted in some survival in areas with appropriate moisture, but the planting in 2010 was quickly eaten, apparently by voles. The site was also being overgrown, bringing into question the restriction on grazing in the preserve. Based on these experiences, the new planting has fourteen sites, seven each in grazed and ungrazed areas, and half of the plants at each site will be protected from herbivores.

Water availability and initial plant size were recorded for each plant. The size of the small mammal population in the area is also being studied. All of these data will be evaluated by faculty and students at Weber State University. It was wonderful to see the plants, which had been sent to the Arboretum at Flagstaff from CREW for acclimatization over the past several years, as robust, healthy plants in soil, ready to take on the dry winds and heat of their new Utah home. Later in the year we should get the first reports on how they are doing.
Rhino Signature Projects
The Peaks and Valleys of Rhino Conservation Research

CREW staff often comments on the brief peaks and unavoidable valleys inherent to careers in wildlife conservation. As scientists, we are accustomed to “going back to the drawing board” when results of a study are not what we anticipated. We learn from those results, adjust our hypotheses and methodologies and conduct another study, a pattern we repeat until we achieve the desired goal. However, in the field of conservation, there are so many confounding factors that are beyond our control that even when the science works, we sometimes find ourselves in a valley. The most recent updates about CREW’s Signature Rhino Conservation Project provide excellent examples of both scenarios. The Indian rhino project is at a peak following a change in methodology that resulted in a tremendous scientific breakthrough. In contrast, wild Sumatran rhinos are in sharp decline and the species is teetering on the edge of extinction despite steady progress with the captive breeding program.

Special Baby Rhino at Montgomery Zoo Produced by Science Pioneered at CREW

On June 5th, the Montgomery Zoo in Alabama announced the birth of a special rhino calf that had strong ties to Cincinnati. Sixteen months earlier, the Montgomery Zoo’s twelve year old female Indian rhinoceros ‘Jet’a had been artificially inseminated (AI) using techniques pioneered by CREW. For Reproductive Physiologist, Dr. Monica Stoops, who leads CREW’s Indian rhino effort, it was a culmination of years of research, teamwork and perseverance. This exciting birth represents the first surviving calf of any rhino species produced by AI in a U.S. Zoo.

Although the female Indian rhino ‘Jeta’ had previously reproduced following natural breeding, AI was requested in 2011 due to behavioral incompatibility with her current mate, ‘Himal’. The ability to integrate AI into the situation eliminated the risks of injuries that may have resulted from aggressive interactions between the pair. The AI technique developed for Indian rhinos at the Cincinnati Zoo was achieved with the help of keeper staff, who conditioned the rhinos to allow the procedure to be performed without the use of anesthetics. Their efforts were rewarded with two term pregnancies at the Cincinnati Zoo in 2007 and 2010, but sadly, neither calf survived. A new approach was needed in order to expand CREW’S AI research to Indian rhinos at facilities that do not have the capacity/facilities to condition rhinos to voluntarily allow hands-on procedures. Working in partnership with Dr. Jack Kottwitz from the Montgomery Zoo, a standing sedation protocol for Indian rhino AI was established. Although logistically difficult, the strategy worked because the Montgomery Zoo’s keepers and veterinary staff were committed to collecting samples and monitoring their rhino closely for signs of behavioral estrus. After the third AI attempt on Jeta using sperm that had been collected at the Wilds and stored in CREW’s CryoBioBank for eight years, the first Indian rhino AI pregnancy outside of Cincinnati was produced. The Montgomery Zoo staff carefully monitored Jeta’s pregnancy over the 480 day gestation period and she successfully gave birth to a male calf named ‘Ethan’.

Ethan’s birth is a significant scientific achievement for CREW, the Montgomery Zoo and the Indian rhino Species Survival Plan since the Indian rhino is an endangered species. This calf demonstrates that sperm banks and AI are valuable tools that can now be integrated into the management strategy for the captive Indian rhino population. With only 60 Indian rhinos in North America zoos and approximately 3,300 remaining in the wild, successful breeding is important for maintaining the genetic diversity necessary to keep a population healthy and self-sustaining. Unfortunately, natural breeding attempts in captive Indian rhinos sometimes result in severe aggression between the male and female. Thus, behavioral incompatibility can hinder the genetic management of the population. AI now offers a method for overcoming this hurdle and infusing genes from non- or under-represented rhinos into the population. Future AI attempts will build upon this novel approach to help not only the Cincinnati Zoo, but other zoos produce Indian rhino calves. (CREW’s Indian rhino program has been supported by several generous private donors, grants from the Morris Animal Foundation and International Rhino Foundation, and most recently, by a National Leadership Grant from the Institute of Museum and Library Services.)

Can Washington D.C. Help Save The Sumatran Rhino?

Despite the successful science that made breeding Sumatran rhinos in captivity achievable, repeatable and transferable and resulted in four Sumatran rhino calves produced at the Cincinnati Zoo and in Sumatra, the species is teetering on the edge of extinction. At a recent Sumatran Rhino Crisis Summit, participants were stunned to learn that there may be fewer than 100 Sumatran rhinos left on the planet. The species is recognized as one of, if not the most endangered large mammal on Earth, and the recent surge in illegal poaching and deforestation due to palm oil are decimating them faster than scientists and conservationists can make incremental progress towards saving them. Since that dire announcement, CREW has engaged in several initiatives that may help change the trajectory for the Sumatran rhino. One that has gained momentum this summer is a collaborative effort with other NGOs (International Rhino Foundation, World Wildlife Fund and SOS Rhino) working in Washington D.C. to recruit help and support from political leaders and the State Department. Ohio Senators Sherrod Brown and Rob Portman as well as Congressman Steve Chabot have all written letters in support of the effort to save this species and a strategy has been drafted and agreed upon by the U.S. and Indonesia regarding necessary next steps to save the last stronghold of Sumatran rhinos on Sumatra. All of these actions (and a little luck) are going to be needed to keep this species from slipping away, but working together, passionate people can and do achieve amazing things.

Ohio’s U.S. Congressman, Steve Chabot, visits Sumatran rhino Harapan

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**Small Cats Signature Project Updates**

**Timing is Everything**

(especially for Artificial Insemination in Cats)

Artificial insemination (AI) is becoming one of the keys to sustaining some wildlife populations in zoos, allowing us to reproduce behaviorally incompatible individuals and use frozen semen for genetic exchange as an alternative to transporting live animals between distant institutions. In cats, one major challenge with AI has been the need to synchronize ovarian activity for the proper timing of insemination. With domestic cats at CREW, we can monitor their reproductive behavior and hormonal profiles over days to weeks to ensure that the gonadotropin injections (needed to induce ovarian follicle growth and ovulation) are administered at the optimal time point. However, with felids housed at other institutions, it is extremely difficult to apply a similar time-sensitive monitoring approach to schedule AI procedures. In a recent study at CREW, we assessed the feasibility of conducting fixed-time AI in domestic cats, using liquid progesterone fed to cats daily for several weeks to shut down ovarian activity prior to the gonadotropin injections. For AI, each female then was inseminated in one oviduct with freshly-collected semen from one male and in the other oviduct with frozen semen from a second male. Most females (6 of 7, 86%) fed the oral progesterone conceived after laparoscopic oviductal AI, producing an average of ~7 fetuses per pregnancy, compared to 6 of 8 (75%) control females becoming pregnant with ~4 fetuses each. We also found that AI with frozen semen produced nearly as many pregnancies and fetuses as seen following AI with freshly-collected semen. These findings suggest that oral progesterone treatment for fixed-time oviductal AI might also be applicable to non-domestic felids housed at other zoological parks, possibly improving our AI success with both freshly-collected and frozen semen in these endangered cat species.

**The Complexity of Comprehending Cheetah Cortisol**

One primary focus of the Cincinnati Zoo’s Cat Ambassador Program (CAP) is to educate the public about the amazing world of felids, including the fastest mammal on earth, the cheetah. Because of their docile demeanor, cheetahs are the one large-sized cat species that can be safely handled as education ambassadors. Although cheetahs may appear to be non-plussed by public exposure, this cat species is known to be susceptible to a number of unusual diseases, with “stress” considered a possible contributory factor in zoos. At the request of Cathryn Hilker, the founder of the Zoo’s CAP, CREW initiated a study to assess the relative “stress” levels of CAP cheetahs compared to other zoo cheetahs maintained on public exhibit or managed at our off-site breeding facility. As an indirect indicator of “stress”, basal and peak levels of adrenocortical hormones (corticoids) were measured in ~450 fecal samples collected serially from 12 individual cheetahs. Keepers of each cheetah population also completed temperament surveys for each cat evaluating 17 different personality traits on a longitudinal scale. Our results showed that CAP cheetahs had similar basal and peak corticoid levels compared to other zoo cheetahs housed on public exhibit and significantly lower levels than found in breeding cheetahs with minimal exposure to humans. Across populations, temperament scores revealed strong correlations between corticaloid levels and traits such as aggression, fearfulness, frequent vocalization and friendliness. These findings indicate that CAP cheetahs, raised from an early age in close contact with humans, have similar (or lower) levels of adrenocortical activity as cheetahs that experience much less direct public interaction. (This study was supported, in part, by the Angel Fund.)

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**CREW ReView**

**Getting to the Root of Genetic Diversity**

In August, CREW staff travelled to the Daniel Boone National Forest in Kentucky to accompany the US Forest Service in their annual survey of the Cumberland sandwort (Arenaria cumberlandensis) experimental outplanting. From the original 77 plants planted in 2005 from CREW’s tissue culture lab, the outplanting has expanded to over 160 plants! On our trip, we collected 35 leaf samples from the experimental outplanting and 150 leaf samples from the two populations of Cumberland sandwort in Pickett State Park in Tennessee, from which CREW’s original seeds were collected in 1994. Using microsatellite markers, the genetic diversity of the outplanting will be compared to samples from the original populations, as well as to samples from tissue culture at CREW and samples that have been thawed after many years of liquid nitrogen storage. So far, we have removed Cumberland sandwort samples that have been preserved in liquid nitrogen in CREW’s CryoBioBank for 7 to 13 years, and several small plants are starting to grow from these. This study, funded in part by a grant from the Institute of Museum and Library Services and also supported by CREW’s Eisenberg Fellowship, should give us some insight into the long-term effects of tissue culture and liquid nitrogen storage on plant DNA, as well as how to maximize our effectiveness in using tissue culture to supplement and re-establish endangered plant populations. Stay tuned for our results!

**CREW’s Waterdog Eggstravaganza**

CREW’s aquatic salamander laboratory supports the only zoo-based captive assurance population of the endangered Black Warrior waterdog. In the first year of the project, CREW scientists developed the life support and husbandry protocols necessary to maintain this species in captivity. In the following two years, the focus has been on stimulating successful breeding and reproduction in three species of waterdogs by administering exogenous hormones and implementing seasonal changes in water temperature and lighting. In 2012, a single black warrior waterdog ovulated and deposited over 50 eggs during three days in April. This year, one Mudpuppy, one Black Warrior and two Gulf Coast waterdogs deposited eggs! Whereas some eggs were not fertile, several eggs from two Gulf Coast waterdogs were fertilized marking the first time this species has successfully reproduced in a zoo setting. A waterdog eggstravaganza! Nesting female waterdogs were kept with their eggs until 33 days post-oviposition, at which time artificial brooding of seven embryos took place. CREW scientists were excited to see the first detectable movement of the embryos at day 55. While never documented before, it was anticipated the larvae would hatch at 60 days, but it actually took a bit longer. From June 17-19, 2013 (day 73-75 post-oviposition), six waterdog hatchlings successfully emerged in CREW’s aquatic salamander lab. CREW scientists can attest to the fact that breeding species with a dedicated aquatic life history, cryptic activity patterns and highly seasonal reproductive nature is not an easy task. While there have been occasional challenges, the waterdogs have been challenging, but well worth the effort. Over the next year, we hope to improve our waterdog breeding, hatching and rearing success and expand it to other species, like the Black Warrior waterdog. (This project is supported, in part, by a gift from Iris de la Mott.)


Lindner Center for Conservation and Research of Endangered Wildlife • Cincinnati Zoo & Botanical Garden

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CREW ReView

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