SUMATRAN RHINO LEGACY GROWS

Roth’s Remarks

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

Early this summer, halfway around the globe, a momentous event took place that had rhino enthusiasts and wildlife conservationists celebrating in unison. On June 23, 2012, a baby rhino was born in Indonesia representing the first Sumatran rhino calf produced outside of the Cincinnati Zoo since the captive breeding program began in 1985. Word of the newborn calf reverberated over the world’s newswires in over 235 articles within 48 hours of the delivery. Little “Andatu” was named for both his wild-caught mother “Ratu” and Cincinnati Zoo-born father “Andalas.” For over a decade, CREW scientists have been working closely with their Indonesian colleagues and hoping for success at the Sumatran Rhino Sanctuary. In 2007, the LA Zoo sent Cincinnati’s first born calf, Andalas, to Indonesia to serve as the breeding male at the reserve. This summer, all those years of international cooperation paid off. When we celebrated the monumental birth of Andalas at the Cincinnati Zoo in 2001, we never imagined he would play such a pivotal role in the survival of his species, and we could not be prouder of what our Indonesian colleagues have achieved. Meanwhile, the wild Sumatran rhino population has plummeted to just 200 individuals, and the tiny captive population of just 11 rhinos may represent our last hope to save this species. The Cincinnati Zoo’s tremendous impact on the Sumatran rhino conservation effort is one of the most compelling examples demonstrating how a zoo can play a key role in saving a species on the very brink of extinction. (The Sumatran rhino program has been supported by grants from the Institute for Museum and Library Services, the International Rhino Foundation and the Mohamed bin Zayed Species Conservation Fund.)
Helping to Secure Southern Oaks

Oaks are one of the most iconic features of the American landscape, but they are not immune to the many modern threats to plant biodiversity, including habitat loss, unsustainable logging, and invasive species. Climate change and disease, particularly Sudden Oak Death, are additional stresses that are clouding the future of these mighty plants. Although there is little information on most of the world’s 500 oak species, the IUCN reports that at least 56 species are endangered. Conservation efforts are challenged by the fact that oak seeds (acorns) are short-lived and that some rare species are hybridizing with common species. CREW’s in vitro methods could assist conservation efforts by propagating true-to-type oaks, as well as providing tissues for cryopreservation. In 2011, CREW Plant Division researchers worked with Botanic Gardens Conservation International (BGCI) to evaluate the use of in vitro methods for propagating four endangered oaks from the southern U.S.: Mapleleaf oak (Quercus acerifolia), Arkansas oak (Q. arkansana), Boynton’s oak (Q. boytonii), and Georgia oak (Q. georgiana).

Young leaves and shoot tips from each species were put into culture, using methods that had been reported for more common oaks. Three of the species responded to the procedures and produced both somatic embryos and shoots in culture. Only tissues from Q. acerifolia did not grow, likely because they were the oldest tissues tested. The shoot cultures initiated from these studies are now being evaluated for rooting potential to complete the propagation cycle and are providing tissue for cryopreservation efforts to help secure the future for these rare U.S. taxa. (This project was supported, in part, by funding from BGCI).
The Vegan Advantage for Semen Freezing and Artificial Insemination in Cats

Vegan diets, which exclude all animal-derived products, have been embraced by a growing number of people for health and ethical reasons. Although cats, as strict carnivores, are not good candidates for veganism, their sperm actually may prefer a vegan environment for cryopreservation. For freezing, cat semen traditionally is diluted in a cryoprotectant solution supplemented with egg yolk, which contains a complex mixture of proteins and lipids that help to preserve sperm cell membranes. However, the precise components of egg yolk can vary considerably from egg-to-egg, and the transmission of disease, such as avian influenza, is a concern, especially with international semen transport. By substituting a plant protein—soy lecithin—for egg yolk, it may be possible to eliminate all animal proteins, reduce disease risk and produce a more consistent cryopreservation medium. In our earlier research at CREW, we found that cat semen frozen in the soy lecithin (SOY) medium had better motility and similar fertility in vitro to semen frozen in the egg yolk (EY) medium. In our most recent study, we compared the fertility of semen frozen in the two media for artificial insemination (AI). Thirteen domestic cats were each inseminated in one oviduct with spermatozoa frozen in SOY and, in the opposite oviduct with spermatozoa (from a different male) frozen in EY. Seven cats (54%) became pregnant, with paternity testing showing that 68% (32/47) of the offspring were produced by sperm frozen in SOY. The high pregnancy percentage after AI with frozen semen and the superior fertility with the ‘vegan’ cryopreservation medium may represent significant advances in our ability to use AI for the global conservation of endangered cats. (Supported, in part, by a grant from the Institute of Museum and Library Services)

The Trouble with Tigers

Historically, artificial insemination (AI) success in tigers has been minimal. At least 60 AI procedures have been attempted in tigers over the past 20 years with only 3 resulting in pregnancies. With such low prospects for producing cubs, AI research with tigers has stalled, depriving zoo curators of an invaluable tool for reproductive management. Because of our recent progress using laparoscopic oviductal AI in small cats, CREW was asked to apply its AI expertise to tigers as well. This past October, we conducted our first tiger AI procedures at the Riverbanks Zoo in South Carolina. Their male Amur tiger was unsuccessful in breeding either of his female companions, and, at semen collection, was found to have a testicular tumor that interfered with sperm production. Using frozen semen samples provided in 2011 by the Riverbanks Zoo, we performed with another unsuccessful breeding pair at the Blank Park Zoo in Iowa. In this instance, high-quality semen was recovered from the male for the AI. Although none of the females conceived, all three did respond well to the hormone treatments with multiple ovulations and adequate progesterone production. We also gained valuable experience in scaling up our small cat methods to perform laparoscopic AI on cats weighing 300+ lbs. This fall, we will embark on a larger tiger AI study with grant support from the Riverbanks Zoo and Minnesota Zoo. Our goal is not to ‘tame the tiger’ but to just improve the success of tiger assisted reproduction to ensure their survival well into the future.

Advances in Acclimatization Assist Avon Park Harebells

In small size often makes it hard to spot amidst the larger plants, deer lichen, and leaf debris in Florida scrublands, but the little legume, Avon Park harebells (Crotalaria avonensis), has been a conservation priority of CREW’s Plant Division, Archbold Biological Station and Bok Tower Gardens for several years. Previously, under an IMLS grant, CREW developed a protocol for propagating this species in vitro, starting with small shoot tips collected in the wild. Since then, samples have been collected from dozens of plants from the three known populations of this species, and there are currently 146 different genotypes in culture at CREW. The plants propagate well, but acclimatizing the rooted shoots to soil and to the harsh heat and strong light of its native habitat has proven challenging. Finally, a step-wise process has been established that appears successful. CREW acclimatizes the plants in closed plastic boxes for one month before sending them to Bok Tower Gardens where they are grown in an incubator with controlled temperature, light, and humidity before being transferred to a greenhouse, where conditions more closely resemble those in the field. CREW has sent over 100 plants to Bok, and in late August, 87 of these plants were transferred to Archbold Biological Station to be used in establishing a population at a protected, wild site. The methods developed at CREW and in Florida for propagating healthy plants of this species are particularly important for at-risk genotypes that may soon be lost in the wild. (This project funded, in part, by the U.S. Fish & Wildlife Service)

Cultivating Carter’s and Cowhorn Orchids for Conservation and Research

Florida is home to a rich and diverse flora under threat from habitat loss and encroachment by invasive species. Two of the state’s endangered species are the strikingly beautiful Carter’s orchid (Basiphyllaea corallicola) and Cowhorn orchid (Cyrtopodium punctatum). Carter’s orchids are native to the increasingly rare pine rockland habitat of Miami-Dade County and the Everglades, while the Cowhorn orchid grows in the swamps of south Florida. Because orchid seeds have no nutrient reserves, in nature they rely on symbiotic fungi to supply them with nutrients for germination. However, by putting the seeds on a nutrient-rich, tissue culture medium, the seeds can be induced to germinate without a fungus. CREW researchers have been using in vitro methods to germinate and propagate these species in collaboration with Fairchild Tropical Botanic Garden (FTBG). Plants of the Cowhorn orchid propagated at CREW are now being used in a study at Florida International University and FTBG on the reproductive biology of this rare orchid and how it compares to that of a related and potentially invasive orchid, Cyrtopodium polyphillum, which has been found in south Florida. The ability to propagate plants of this endangered orchid makes research like this possible without disturbing wild plants. CREW’s propagated Carter’s orchids are being sent to FTBG for further growth and potential outplanting. In both cases, CREW’s in vitro methods are facilitating the study and preservation of these floral gems for future generations. (This work supported, in part, by a grant from FTBG)
CREW scientists work to unravel the mystery of low reproductive success in captive polar bears

As with humans, one of the first things fertility experts must determine when wildlife species are having trouble reproducing is where the problem lies. Is it “his fault” or “her fault”? After answering that question, they can prescribe the appropriate remedies that hopefully will result in the successful birth of healthy offspring. Such is the situation with polar bears and CREW scientists. Polar bears in zoos have not been reproducing well, in part, because of a previous moratorium on captive breeding that resulted in a skewed demographic towards aged animals with no reproductive experience. However, even younger or previously proven male-female pairs are producing far below expectations. Therefore, five years ago, CREW researchers started investigating the reproductive biology of polar bears. However, studying reproduction in polar bears can be much more challenging than in humans because most bear data must be collected non-invasively. After years of assessing fecal hormone metabolites, CREW researchers finally have some answers, and of course, a lot more questions.

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**HER SIDE:**

After validating the appropriate assays for monitoring female polar bear reproductive activity, CREW scientists conducted a large-scale, two year study involving over 20 female polar bears in U.S. and Canadian zoos. Two reproductive hurdles commonly identified in zoo animals are 1) lack of female reproductive cyclicity and 2) behavioral incompatibility between genetically paired individuals. Results from CREW’s research demonstrated that neither of these is the primary challenge with polar bear reproduction. Most of the female bears exhibited follicular activity, and the majority of those paired with males mated at least once during the natural breeding season. However, only four females gave birth, for a total of five litters over two years. Unlike wild polar bears, captive females appeared to experience a much higher frequency of pseudopregnancy. Pseudopregnancy is a pregnancy-like state that the females enter after mating, ovulating, but not conceiving. It is estimated that pseudopregnancy occurs in less than 10% of wild female polar bears, whereas over 50% of the female bears in our study appeared to experience this condition based on their hormone profiles and the lack of cub production. However, some of these bears could have conceived and lost their pregnancies early in gestation. Unfortunately, fecal hormone metabolite analyses do not allow us to distinguish between true pregnancy and pseudopregnancy. Additional studies are underway to develop a definitive pregnancy test for this species. Meanwhile, our attention turned to the male bears to determine if they exhibited any signs of reproductive deficiencies that could lead to infertile matings with the females.

CREW scientists recently completed a three year study in which fecal testosterone concentrations were monitored in 14 adult male polar bears from 13 zoos ranging in location from Alaska to Arizona. The primary goal of the study was to verify that, like their wild counterparts, male bears are experiencing increases in testosterone during the breeding season. Furthermore, because captive bears generally live at lower latitudes and warmer climates relative to their wild cousins, we wanted to determine if those environmental variants were affecting testosterone production. Results allowed us to confirm that testosterone is significantly higher during the breeding season (Jan-May) compared to the rest of the year. Even males that did not breed or were not housed with females experienced seasonal testosterone increases, albeit with lower concentrations than in breeding males. Testosterone was lowest in the younger males, peaked at ~10-18 years, and then decreased in bears of older ages. Overall, latitude did not affect testosterone concentrations during the breeding season, although males at lower latitudes tended to exhibit lower testosterone during the summer months. It is unlikely that decreased testosterone in the summer would inhibit male fertility because the breeding season occurs at the end of the coldest months when testosterone concentrations were similar among all latitude groups. In conclusion, captive males experience seasonal fluctuations in testosterone appropriate to the breeding season and comparable to their wild counterparts. There is no evidence of diminished seasonal cues or seasonal asynchrony between the sexes that could lead to low conception rates. Results from this study did not reveal any aberrations in testosterone production that would negatively affect the fertility of captive male polar bears.

**SORRY, GIRLS, BUT IT LOOKS LIKE THE PROBLEM LIES WITH YOU.**

(Supported, in part, by grants from the Shumaker Family Foundation and the Association for Zoos and Aquariums’ Conservation Endowment Fund)
Probing Potto Procreation

The potto is an arboreal, nocturnal primate, which has been sparsely studied. The Cincinnati Zoo & Botanical Garden is one of only four zoos within the Association of Zoos and Aquariaums exhibiting pottos, and our propagation program has produced 15 baby pottos since 1974. CREW scientists recognize the unique research opportunity provided by having such a successful breeding population of pottos. Through fecal hormone monitoring, ultrasound, and behavior, CREW scientists are gathering basic reproductive information on this most primitive of primate species. By tracking behavioral changes in a young female, we confirmed that the transition from adolescence to sexual maturity occurs at 18 months of age. Similarly, we determined that adult females exhibit estrous cycles every 30 to 75 days. Wild pottos are known to be seasonal breeders, but in captivity, maintained on a constant photo-period of 12 h light and 12 h dark, females continue to cycle and males maintain consistent testosterone levels regardless of season. Two pregnancies that resulted in the birth of live young were confirmed by ultrasound exams conducted 15 weeks post breeding and were monitored through fecal hormone analyses. Results from these scientific approaches, combined with behavioral observations of breeding, indicate gestation length for the potto averages 170 days. Our findings from this study helped establish fecal hormone monitoring and ultrasonography as effective tools for both assessing reproduction in pottos and learning about their basic biology. Furthermore, all of this information will provide a valuable foundation for future potto research.

Evidence for Delayed Implantation in Red Pandas

Despite their popularity in zoos, very little is known about the reproductive physiology of red pandas. Previous studies reported that the duration of pregnancy in red pandas is variable, ranging from 98 to 145 days. This inconsistency could be explained by a reproductive phenomenon called delayed implantation, in which early embryonic growth is arrested at the blastocyst stage until environmental cues stimulate resumption of development. In species such as polar bears and otters, the occurrence of delayed implantation (or embryonic diapause) helps to ensure that offspring are born at a time of year favorable to their survival. CREW researchers began working with the Cincinnati Zoo’s amicable red pandas in an attempt to learn more about this enigmatic physiological state. Female red panda, Bailey, was trained to stand voluntarily for transabdominal ultrasonography, and exams were performed once or twice weekly throughout the breeding season and pregnancy. A fluid-filled uterus is the first sign of pregnancy and can be detected by ultrasound approximately two weeks after a fertile mating in species that do not undergo embryonic diapause. With Bailey, uterine fluid was not detected until 48 days post-mating, indicating a delay in pregnancy progression. It took another 23 days before a fetus was observed, but then gestation progressed quickly, and Bailey gave birth to a healthy cub 107 days after mating (but just 59 days following first detection of uterine fluid). Although more work is needed to elucidate the factors controlling embryonic diapause, this study provides the first visual evidence of possible delayed implantation in the red panda.

Ultrasound exam to diagnose pregnancy in a potto.

Diets and Bladder Cancer – a Possible Linkage in Fishing Cats?

In humans, we know that diets can influence the development of some cancers, such as colon cancer and bladder cancer. But do diets have similar effects on cancers in wildlife species? P&G Wildlife Conservation Scholar Emily Marshall is attempting to answer that question with her ongoing research in the fishing cat (Prionailurus viverrinus). As the name implies, fishing cats typically eat a lot of fish in their natural wetland habitats in Southeast Asia in addition to smaller quantities of shellfish, rodents and birds. In contrast, fishing cats in zoos usually are fed the same diets (ground beef supplemented with vitamins/minerals) as other cat species and receive very little fish. However, fishing cats differ from all other cat species in experiencing a very high prevalence of bladder cancer as they age, and we suspect that diets are involved. In collaborative research with Ohio State University, P&G Pet Care and CREW, Emily compared nutritional parameters in blood samples from fishing cats with bladder cancer and those without any evidence of this disease. Similar to findings in humans, fishing cats with bladder cancer had higher levels of saturated fats than non-affected cats, reflecting the composition of their beef diets. In a follow-up study, Emily is analyzing nutrient levels in domestic cats and fishing cats maintained on beef versus fish diets. Based on Emily’s initial results, North American zoos will be modifying their fishing cat diets to contain > 75% fish, and hopefully help to decrease the occurrence of bladder cancer in this endangered cat species.

P&G Wildlife Conservation Scholarship Program

In 2011, a summer scholarship program was established at CREW with the support of Procter & Gamble Pet Care to give hands-on scientific training to veterinary students interested in pursuing wildlife conservation research as a career. In a partnership between CREW and the Ohio State University’s College of Veterinary Medicine, the P&G scholarship provides financial support to two veterinary students each year to conduct wildlife research studies with mentoring by OSU and CREW scientists.

Helping Rhinos Adjust to Change

Similar to other animals, rhinos prefer consistency in their daily routine. When relatively small changes are made, it’s easy for them to adjust. However, bigger changes, such as moving to a new zoo, can be quite stressful. We all know how hard it is to be the new kid on the block. Regardless, moving rhinos between zoos is necessary to fulfill breeding recommendations made between genetically diverse pairs. How each rhino adapts after transport varies depending on individual temperament and prior experience. For many rhinos, adaption occurs without much fanfare; however, some have a harder time adjusting. For those more temperamental individuals, enhanced secretion of the stress hormone cortisol can lead to a decrease in reproductive efficiency, which is counterproductive to the original transport goals. Anni Benco, a 2012 P&G Wildlife Conservation Scholar, joined CREW scientist Dr. Monica Stoops and Ohio State University professor Dr. Carlos Pinto in a study aimed at determining if a long-acting neuroleptic can help tentative rhinos adapt to new surroundings. Neuroleptics are frequently used during the transport of rhinos, but their therapeutic effects wear off shortly after arrival. Anni examined if daily administration of these drugs during the first few months that a rhino is adapting to new exhibits and/or social partners could prove useful. By helping rhinos adjust to change, Anni’s study furthers CREW’s effort to optimize the welfare of these animals in captivity.

Adjust to Change

Diets and Bladder Cancer – a Possible Linkage in Fishing Cats?

Emily Marshall with Minnow, the fishing cat

Evidence for Delayed Implantation in Red Pandas

Evidence for Delayed Implantation in Red Pandas

Helping Rhinos Adjust to Change

P&G Wildlife Conservation Scholarship Program

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CREW Scientific Highlights

PEER-REVIEWED PUBLICATIONS


Kaelin CB, XU LZ, Hong DA, VA KA MC. 2012. Micropropagation, cryopreservation, and support services. Cost: $300.

CREW ReView

Our sincere gratitude to the following who gave $100 or more in 2011:

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CREW Awarded Prestigious National Leadership Grant

CREW recently received a highly regarded federal grant from the Institute of Museum and Library Services (IMLS). This prestigious award of $461,808 over three years will support the Zoo’s Endangered Plant Signature Project. Specifically, CREW’s Plant Division will conduct a study that will provide critical information for botanical gardens in the U.S. and worldwide to aid in developing strategies and methods for cryopreservation as a tool for plant conservation. The research team will assess the viability of over 980 samples from CREW’s CryoBioBank, including seeds, spores, pollen, and tissues from 178 species, many among the nation’s most endangered, that have been stored up to 24 years in liquid nitrogen. Current viability and genetic integrity will be determined and used to evaluate the effectiveness of the methods used at the time of banking, as well as effects of tissue, age, genotype, species, and storage location. This award provides further evidence that CREW is considered one of the nation’s visionary leaders in the conservation of endangered plants.

www.myActions.org – the Social Network for the Planet

In today’s world of social network sites that allow individuals to connect, share stories, pictures, real-time events, locations and just about anything about themselves, it is truly refreshing to learn of a social networking site that is actually benefitting the planet. The site: www.myActions.org encourages individuals to engage in green actions (reduce, re-use, recycle) in their daily lives, initially as a fun, competitive way to earn points, win cards, and raise funds for a select conservation organization. However, the ultimate goal of the site is to gradually change everyday behavior of individuals so that these actions are performed automatically by hundreds of thousands of people every day. The Cincinnati Zoo was thrilled to be one of the first conservation organizations chosen as a beneficiary of the website, and all funds raised during a three week period in August went directly to support CREW’s polar bear project. When www.myActions.org really catches on and becomes successful on a global scale, the ultimate “winner” will be earth itself and all of the life it currently supports.

Remembering to turn off the water while you brush your teeth, starting a neighborhood carpool, setting the AC up 2 degrees – it all matters. Visit www.myActions.org today and make your impact count!