



## Museums for America

Sample Application MA-30-18-0461-18  
Project Category: Collections Stewardship

### Cincinnati Zoo and Botanical Gardens

Amount awarded by IMLS:	\$244,216
Amount of cost share:	\$364,807

Attached are the following components excerpted from the original application.

- Abstract
- Narrative
- Schedule of Completion

Please note that the instructions for preparing applications for the FY2019 Museums for America grant program differ from those that guided the preparation of FY2018 applications. Be sure to use the instructions in the [FY2019 Notice of Funding Opportunity](#) for the grant program and project category to which you are applying.

## Abstract

The Lindner Center for Conservation and Research of Endangered Wildlife (CREW) at the Cincinnati Zoo & Botanical Garden is a global leader in research and conservation efforts with endangered wildlife species. Dr. Erin Curry, CREW Reproductive Physiologist, and Dr. Terri Roth, Director of CREW and the Zoo's Vice President of Conservation & Science, seek funds from the Institute of Museum and Library Services Museums for America grant program to improve collections stewardship of zoo polar bears (*Ursus maritimus*) by conserving genetics, enhancing reproduction, and improving animal welfare. The captive population of polar bears is in decline globally and, in the United States, there remain only 66 individuals in the nation's zoos. A low reproductive rate is the principal threat to the collection and, without efforts to improve propagation and breeding management strategies, while preserving valuable genetic material, it is entirely plausible that polar bears will not exist in zoos by the year 2050. In 2013, CREW identified polar bears as one of its four *Signature Projects*, which are focused, long-term programs in which significant impact can be made.

Zoos are striving to develop a self-sustaining population through strategic breeding recommendations; however, few cubs are born each year despite the fact that most bears of reproductive age are paired for breeding. Additionally, a shortage of males is leaving many females of prime reproductive age without a partner for natural mating. Working in collaboration with the polar bear Species Survival Plan (SSP) and 33 organizations in the United States that house polar bears, scientists at CREW are poised to address the reproductive challenges faced by this iconic species to ensure their presence in zoos for years to come. Over the three-year funding period, they propose to: 1) collect and cryopreserve semen so that irreplaceable genetic material will not be lost; 2) resolve the uncertainty around the timing of sexual maturation in this species using non-invasive fecal hormone monitoring so that valuable years are not wasted prior to pairing bears for mating; and, 3) employ a novel, non-invasive pregnancy test based on comparative fecal proteome analysis to better manage the collection and provide enhanced animal welfare.

Through their solid research record and growing expertise, CREW scientists have become recognized leaders in the field of polar bear reproduction and are well qualified to conduct the proposed collection stewardship project. To address the significant knowledge deficits that obscure understanding of polar bear reproductive physiology, they began studying polar bear reproduction in 2007. Since then, they have monitored 63 polar bears at 30 North American institutions to better understand their reproductive processes, including female cyclicity, male reproductive seasonality, and the long-term effects of contraceptives. They have established much needed methodologies for semen collection and cryopreservation and performed the world's first polar bear artificial insemination in 2012.

Completion of this project will greatly enhance the genetic viability and sustainability of polar bear collections maintained in the Nation's zoos. Semen collection and cryopreservation of valuable males will ensure that their genes will not be lost if they fail to reproduce naturally or die unexpectedly. Because 35% of females of reproductive age are without males, semen collected as part of this endeavor will provide a chance of pregnancy for females that do not have a male to breed. Determining the precise age of sexual maturation will drive breeding recommendations and is likely to result in females producing an additional litter or two in their lifetimes. Finally, utilizing a novel, non-invasive pregnancy test based on a fecal protein biomarker will enable better management of both pregnant and non-pregnant individuals and will provide insight into where the reproductive process is failing in this species. Success will be measured by the number of semen samples collected and cryopreserved, the number of juveniles monitored for sexual maturation, and the employment of a novel method of diagnosing pregnancy in both pregnant and non-pregnant individuals. The ultimate measure of success will be the continued survival of polar bears in zoological collections.

## Narrative: Collections stewardship of captive polar bears

### 1. Project Justification

The captive population of polar bears (*Ursus maritimus*) is in decline globally and, in the United States, there remain only 66 individuals in the nation's zoos. A low reproductive rate is the principal threat to the collection: of the 66 bears, only 17 females and 12 males are considered to be of reproductive age and, despite the fact that most bears are observed breeding, only 6 females and 4 males have produced cubs during the last 9 years. Due to a long inter-birth interval and federal restrictions prohibiting the importation of bears from the wild, only 10 surviving captive-born cubs have been added to the population during that time period, which does not compensate for the adult mortality rate during the same interval. Without improved reproductive rates, it is entirely plausible that polar bears will not exist in zoos within two to three decades. Already, the demand for polar bears to fill zoo exhibits cannot be satisfied, as the current population number is only just above half (53%) of the total holding capacity of Association of Zoos & Aquariums (AZA)-accredited institutions. Additionally, a shortage of males is leaving approximately 35% of females of prime reproductive age without a partner for natural mating.

The Lindner Center for Conservation and Research of Endangered Wildlife (CREW) at the Cincinnati Zoo & Botanical Garden seeks a Museums for America Grant in the Collections Stewardship category from the Institute of Museum and Library Services in the amount of \$245,908 over three years to address the IMLS agency-level goal of supporting exemplary stewardship of collections, in this case- polar bears, and the use of technology to facilitate discovery of knowledge to address reproductive challenges in this species. If awarded, these funds would support the conservation and improve management of all polar bears in North American zoological collections, as well as the breeding pair in our own Zoo, ensuring the species' existence for future generations. Specifically, we propose to:

- Collect and cryopreserve semen so that irreplaceable genetic material will not be lost and will be available for the artificial insemination (AI) of females that are without mates;
- Determine the sexual maturation in juveniles using non-invasive fecal hormone monitoring so that valuable years are not wasted prior to pairing bears for mating; and,
- Deploy new technologies based on comparative fecal proteome analyses to diagnose pregnancy, which would enable better management of both pregnant and non-pregnant individuals.

CREW is uniquely qualified to spearhead these initiatives. In 2007, to address the significant knowledge deficit that obscures our understanding of polar bear reproductive physiology, scientists at CREW began studying polar bear reproduction. Using longitudinal non-invasive fecal steroid metabolite analyses, we have monitored 63 individual polar bears at 30 North American institutions to better understand their reproductive processes, including female cyclicity<sup>1</sup>, male reproductive seasonality<sup>2</sup>, and the long-term effects of contraceptives.<sup>3</sup> (Citations for all references can be found in Supporting Documents.) Recently, we have established methodologies for semen collection<sup>4</sup> and cryopreservation. We have identified polar bears as one of CREW's four *Signature* projects, which are priority areas of long-term commitment where we recognize our impact will be significant (as described in the Organizational Profile, the Strategic Plan Summary, and the Letter of Commitment in Supporting Documents). Through our solid research record and growing expertise, we have become recognized leaders in the field of polar bear reproduction and are committed to the conservation of this species. The project goals and activities are in direct alignment with CREW's Strategic Plan and Mission Statement "Saving Species With Science<sup>®</sup>". Taken together, we are expertly poised to address the critical needs of this species to ensure its presence in zoos for years to come.

### Background and Challenges to be Addressed

Polar bear exhibition in zoos dates back to the first wild-caught individual maintained at the Philadelphia Zoo in 1876. Over the next century, the numbers of polar bear cubs born in North American zoos increased and the captive population reached its peak of 229 individuals in 1975 as a result of both increased births and wild

imports.<sup>5</sup> However, due to limited space and the desire to provide larger, more naturalistic exhibits, the demand for polar bears declined. In addition, animal managers were advised against producing cubs, so many bears were sterilized or contracepted.<sup>6</sup> Over the past decade, the zoo polar bear population has been decreasing by approximately 1% each year, while the national demand for exhibit bears now is increasing, but can no longer be met.<sup>6,7</sup> Concurrently, threats facing wild polar bears gained national and international attention, leading to the 2008 official listing of polar bears as a species threatened with extinction by the U.S. Fish & Wildlife Service.<sup>8</sup> Under current regulations, bears cannot be imported from the wild (with few exceptions) or from other countries, so efforts must focus on conserving genetic material and improving management strategies to increase reproductive rates of the captive collection.

All polar bears residing in U.S. zoological institutions are managed cooperatively by a Species Survival Plan (SSP), which aims to maximize the number of cubs born per year and ensure genetic diversity of the population through strategic breeding recommendations based on pedigree. Even though nearly all captive polar bears of reproductive age are paired for breeding, the number of litters produced in recent years is in stark contrast to the numbers observed when the population was at its peak 40 years ago. We currently average 2.0 litters per year versus 9.7 litters per year as seen previously. CREW's recent analysis of the polar bear studbook, which records census data back to the 1880s, revealed that the number of litters produced per year is correlated directly with the number of adults in the population.<sup>5</sup> The existing population numbers are just 26% of those of the population peak in the 1970s, indicating that the population can rebound and be maintained if corrective measures to improve reproduction and increase numbers are initiated. The most recent Population Analysis & Breeding Transfer Plan<sup>9</sup> states that increasing the number of births above two per year would allow the population to increase towards its target size, so even small improvements will be beneficial to the population.

Despite the popularity of this species, little is known about its unique reproductive physiology, which makes managing the collection for breeding and addressing potential causes of reproductive failure challenging. There are uncertainties surrounding the timing and factors influencing sexual maturity in this species and there is no method to diagnose pregnancy non-invasively. Polar bears exhibit a unique medley of reproductive phenomena, including seasonal breeding, induced ovulation, embryonic diapause and pseudo-pregnancy, rendering their reproductive processes difficult to study. Females breed in the springtime and, if an embryo(s) forms, it will enter an arrested growth state (also known as embryonic diapause or delayed implantation) until uterine attachment/implantation in the late summer/early autumn. It is impossible to determine whether a female is truly pregnant or exhibiting a pseudo-pregnancy until she produces cubs (or not) in November or December. Consequently, the window to assess the previous year's reproductive success is narrow, as females without cubs will begin cycling as early as January, so inter-institutional transfers of individuals that fail to produce cubs are difficult to plan logistically without compromising the welfare of a potentially pregnant female. To address some of the challenges associated with managing this species, our proposed project will aim to:

***Preserve valuable genetic material:*** Each year, an older male or two in the population dies and, if he has not sired cubs, his genes are lost permanently. Preserving sperm from these individuals is essential to avoid further permanent loss of genetic diversity; however, the traditional method of semen collection from wildlife, electro-ejaculation, has been relatively unsuccessful in polar bears. Our previous endeavors<sup>4</sup> included the evaluation of a novel, minimally-invasive method of semen collection in this species, which has resulted in an impressive 92% success rate (11 samples/ 12 attempts). For the first time, the creation of a genetically diverse polar bear sperm bank to store these valuable genes is possible. Of equal significance, we have demonstrated that sperm collected and cryopreserved using our technique exhibit up to 65% motility post-thaw, which is suitable for artificial insemination (AI) procedures. A shortage of males has left ~35% of females of reproductive age without a breeding partner, and AI offers their only chance at pregnancy.

***Improve collection management:*** It is likely that current management strategies undervalue the early fertile years of an individual's reproductive lifetime. Polar bears in captivity generally are recommended for breeding starting around the age of 5 or 6 and the average age at first parturition is 9 years.<sup>5</sup> However, there very recently have been reports of wild males as young as 2 siring cubs (unpublished data). If captive bears become sexually mature at the same age as those in the wild, it is likely that we are overlooking valuable breeding opportunities.

With so few cubs born each year and a 3-year interbirth interval, it is vital that every sexually mature individual be paired for breeding to maximize the number of offspring produced during a female's reproductive lifetime.

The ability to diagnose pregnancy would allow both pregnant and non-pregnant individuals to be managed appropriately. Females that are suspected pregnant often are secluded from males in denning areas in well-intentioned efforts to mimic natural conditions. An accurate pregnancy test would prevent weeks of imposed denning of non-pregnant individuals, thereby improving the care of the animals in our collections, enhancing animal welfare, and ensuring that more females are not in dens, but instead are available for public viewing during the autumn and winter months. Additionally, non-pregnant females or males that successfully sired cubs could be moved to other institutions well before the next breeding season, which would provide adequate time for mandatory quarantine periods and controlled introductions to a new breeding partner- this would ensure that a valuable breeding season would not be missed.

### **Project Beneficiaries**

A substantial number of zoos, organizations, and individuals will benefit from the proposed activities. Well-established collaborations with other zoological institutions ensure that all zoos that currently maintain or hope to maintain polar bears in their collections may benefit. At present, [33 zoos](#) dispersed throughout the U.S. participate in the polar bear SSP and have dedicated facilities and resources to house and exhibit polar bears. However, if the number of bears continues to decrease, many institutions will be forced to phase out this species from their collection plans. It is quite possible that polar bears may not exist in zoos by 2050 if current reproductive trends persist. Additionally, the use of semen cryopreserved in CREW's CryoBioBank will be made available to AZA-accredited institutions for AI procedures at no cost. The [Polar bear SSP](#) recognizes the value of these initiatives and has fully endorsed the proposed activities (see Supporting Documents). The SSP will benefit from the project results because they will be provided with crucial information regarding sexual maturity and pregnancy status that is unattainable currently. This information will guide SSP management decisions to maximize reproductive success. Because the existence of the SSP relies on successful reproduction to sustain the collection, it is essential that captive polar bears exhibit an increase in reproductive rates. From 2014-2017, CREW received requests from individuals involved in the care of polar bears in six [other countries](#). The ability to share knowledge internationally has multiple benefits and would be useful to every institution in the world that houses polar bears. [Polar bears themselves](#) would benefit, through improved animal welfare. Both pregnant and non-pregnant females would be managed appropriately, promoting improved standards of care. Additionally, the technologies, advances in methodologies, and findings resulting from this project may have much broader application to [multiple species](#) in zoological collections that exhibit delayed implantation and/or pseudo-pregnancy, such as exotic cat species, otters, and red pandas, to name just a few. [Arctic field biologists](#) have requested CREW's expertise in semen collection methodologies and pregnancy diagnoses in this species. Not only will the proposed project preserve gametes for future use in zoological collections, but the characterization of semen parameters, such as sperm concentration, motility, and morphological abnormalities of captive bears will provide baseline information useful for studying the impacts of environmental pollutants on the fertility of wild bears. A pregnancy test would be useful in the field for predicting population growth and would allow scientists to determine the number of lost pregnancies resulting from poor maternal body condition. Most people will never see a polar bear in the wild, but approximately 181 million people visit zoos each year, more visitors than NFL, NBA, NHL, and MLB annual sports attendance combined.<sup>10</sup> Although this proposal is not being submitted in the Learning Experience category, a regular activity of CREW scientists is to convey messages of conservation to broad audiences and to train the next generation of scientists. [Zoo visitors](#) consistently rank polar bears as one of their favorite animals to visit- they enjoy seeing this charismatic species in person. Zoo polar bears also educate the public by acting as arctic ambassadors for their wild cousins, helping to communicate the messages of conservation and climate change to broad audiences in the zoological setting. Finally, CREW trains [post-doctoral fellows, students, interns, and volunteers](#) at all levels. An important component of this proposal is to invest in the professional development of the next generation of museum professionals by funding a Postdoctoral Fellowship, which is of special interest to IMLS for FY18.

### **How will your project advance your institution's strategic plan?**

All activities described in this proposal are strongly supported by the Cincinnati Zoo & Botanical Garden's strategic plan (see Strategic Plan Summary), which is based on the central strategic objective of "inspiring every visitor with wildlife every day." Specifically for CREW, the project directly addresses three of four major strategies, specifically by: emphasizing a CREW *Signature* Project (polar bears); supporting an emerging project (determining the point of reproductive failure); and, applying CREW expertise to improve management and propagation of targeted species (semen collection and monitoring juveniles in the population for better management).

### **Alignment with IMLS Museums for America Program**

The proposed project is in seamless alignment with the IMLS category of Collections Stewardship. Its activities will result in: the exemplary management, care, and conservation of museum collections; long-term preservation of materials and specimens through cryopreservation of valuable genetic material, and; skill-building and capacity-expanding programs by implementing state-of-the-art technologies to improve diagnostic prowess that will improve collection management. We are addressing high-priority issues in a high-profile species by using advanced technologies to better care for and better manage captive polar bears. These activities will ensure that museums may continue to exhibit this charismatic species for years to come.

## **2. Project Work Plan**

### **Specific Activities**

Our proposal includes three interconnected, independent activities with the overarching goal of providing exemplary collections stewardship of captive polar bears and using technology to facilitate discovery of knowledge to ensure better management of the collection. Specifically, as outlined in the IMLS Collections Stewardship description, the activities focus on "planning for the management, care, and conservation of collections." The timing of each activity is described in the text for each and the sequence is shown in the attached Schedule of Completion.

#### ***Activity 1: Collect and cryopreserve semen from male polar bears to fully establish a polar bear sperm bank***

Because traditional methods of collecting semen are ineffective in polar bears, neither zoo nor field scientists have collected sperm from this species until recently. Medetomidine, an  $\alpha 2$ -adrenergic agonist commonly used to anesthetize captive polar bears, has side effects that facilitate semen collection: it stimulates epididymal receptors, causing semen to enter the urethra while concurrently stimulating receptors in the neck of the bladder, preventing urine contamination. Following the administration of anesthesia, a sterile catheter is threaded into the urethra and semen pools within it. The catheter containing the semen then is slowly retracted. The entire procedure takes less than five minutes and can be performed concurrently with other medical procedures. Scientists at CREW assessed the feasibility of collecting semen via urethral catheterization from male polar bears anesthetized with medetomidine<sup>4</sup> (0.025-0.060 mg/kg). They recovered semen containing motile spermatozoa in 11 of 12 procedures (91.7%), making the establishment of a polar bear sperm repository a realistic possibility. Of equal significance, it was demonstrated that sperm collected and cryopreserved via CREW's technique exhibit up to 65% motility post-thaw, which is suitable for AI procedures (unpublished data). Over the duration of the grant, semen collection attempts will be performed on sexually mature male polar bears at zoos across the nation. Any semen recovered will be assessed for motility and morphology, extended, cryopreserved, and stored in CREW's CryoBioBank<sup>®</sup>.

*Evaluation and performance measurements:* Two-to-three semen collection and cryopreservation procedures will be performed per year. This would result in a minimum of 60-120 straws of cryopreserved semen over three years, which would be enough to inseminate every female housed without a male for the next few decades if necessary. Progress will be evaluated at the end of each calendar year by comparing the number of collection procedures performed and the number of sperm samples cryopreserved to the proposed number.



*Potential pitfalls:* Based on past years, we expect to collect sperm from at least 2-3 males per year of the project. Because collections are conducted at annual physicals or pre-shipment examinations, there is the possibility that we will have fewer opportunities to collect samples some years. Furthermore, every collection attempt may not be successful and each sample collected may not exhibit adequate sperm parameters for freezing; however, based on our opportunities to collect samples over the past several years and the high rate of success (nearly 92%), we expect to retrieve an adequate, freezable sample at most collection attempts.

### ***Activity 2: Monitor sexual maturation in polar bears for improved management***

All fecal steroid extraction techniques and enzyme immunoassays required for the completion of this activity are in use currently in our lab. Because there are few young individuals in the population (due to low birth rate and low importation rate), individuals have been recruited opportunistically from 17 different institutions since 2008. At least five year-long profiles from bears ranging in age from 1.0-6.9 years of age will be generated by the end of 2019. Any cubs born in 2017-2018 also will be monitored.

*Sample collection, hormone extraction, and enzyme immunoassay:* Approximately 85% of the fecal samples required for the completion of this activity already have been collected (see Supporting Documents), and roughly 60% of those samples already have been analyzed as a result of a generous private donation. Concentrations of progesterone and testosterone metabolites in each sample are measured in duplicate using established enzyme immunoassay techniques<sup>11</sup> to characterize female cyclicity and seasonal increases in testosterone in males.

*Evaluation and performance measurements:* Our goal is to perform longitudinal fecal hormone monitoring to decipher the fluctuations in fecal steroid metabolites throughout sexual maturity in both male and female polar bears. Each institution, as well as the SSP, will receive a report containing the hormone profile of their bear(s) twice per year, which will guide management decisions. Following cessation of the monitoring, a specific recommendation will be made to the SSP regarding the appropriate age individuals should be managed for breeding.

*Potential pitfalls:* Since ~85% of the samples have already been collected and the required assays currently are in use in our lab, we do not anticipate any major challenges with this activity.

### ***Activity 3: Utilize fecal proteomics for pregnancy diagnostics***

We previously identified several fecal proteins that are higher in pregnancy when compared to pseudopregnancy<sup>12</sup>. Our first attempt to utilize one of these proteins (transthyretin) initially produced very promising results, but in the end, was not diagnostic for pregnancy due to low protein abundance; however, there are at least four additional proteins that merit investigation. The two most promising candidates are: carboxypeptidase B, which was detected in higher concentrations than transthyretin, and immunoglobulin heavy chain variable region, which was much more abundant in pregnant versus pseudopregnant samples ( $p < 0.0002$ ).

*Pregnancy assessment:* The template for fecal protein extraction and quantification via EIA and gel electrophoresis has been established at CREW through our work with transthyretin. The next step proposed herein, is to apply the new technology of these established protocols to assess the two proteins exhibiting a greater fold-change in concentration between pregnant and pseudopregnant bears. Specifically, we will assess the concentrations of these proteins in samples collected longitudinally from pregnant and non-pregnant bears and then choose the most definitive pregnancy indicator to determine when, exactly, pregnancy is failing in some individuals. These activities will provide animal care staff with information necessary to evaluate and improve management strategies in relation to reproductive failure.

*Evaluation and performance measurements:* All fecal samples required for the completion of this project already have been collected and are stored at CREW. By the end of the first year, assay development will be well underway. During the second and third years, protein excretion trends will be characterized in bears of known pregnancy status and those failing to produce cubs. Progress will be evaluated at the end of each year by matching accomplished activities to their predicted timeline.

*Potential pitfalls:* It is possible that some antibodies may fail to cross-react with polar bear proteins; in this case, custom-designed antibodies may be generated or detection methods not reliant on antibody-binding may be utilized. Admittedly, it is also possible that none of the previously identified proteins<sup>12</sup> will be a suitable biomarker of pregnancy since that initial work was based on few individuals; however, because those initial results were very convincing and a pregnancy test is greatly needed to improve the welfare of this species, this project warrants attention. The ability to diagnose pregnancy and identify the point of reproductive failure would be a game changer in the day-to-day management of polar bears as well as in collection planning. The PIs and research associate have extensive experience in proteomics and in the development of EIAs, so no challenges are anticipated from a technical standpoint. Additionally, all of the samples already have been collected and are stored at CREW.

## **Project Management and Financial, Personnel, and Other Resources**

### ***Management and personnel:***

***CREW:*** Dr. Erin Curry, Reproductive Physiologist, (75% time commitment) will serve as Project Director. Dr. Curry was hired at CREW in 2011 and currently oversees CREW's Polar Bear *Signature Project*. She also is the reproductive advisor to the newly formed Polar Bear Research Council. Dr. Curry is highly experienced in EIA and protein analysis techniques, including protein isolation, quantification, and antibody-based detection systems. She has proficiency in semen collection and assisted reproduction techniques in multiple species, with a focus on polar bears. Dr. Curry will ensure proper oversight concerning all administrative activities and will manage relationships among CREW, the polar bear SSP, and the participating institutions. She will provide overall project supervision and oversight of assay development and validation, data analysis, summaries, and publications. Dr. Terri L Roth, Director of CREW and the Zoo's Vice President of Conservation & Science (5% time commitment), is an internationally recognized expert in wildlife reproduction and will assist with project planning, hormone analysis interpretation, and dissemination of results.

In alignment with the special interests of IMLS in FY18, to invest in the professional development of museum staff, we are requesting funds to create a Postdoctoral Fellow position. A Postdoctoral Fellow will be identified and hired for a 2-year term, will dedicate 95% of his/her time to the proposed activities, and will be mentored by CREW senior scientists. Endocrine Laboratory Research Associates, Research Technicians, and an established group of CREW Endocrine Lab interns and volunteers will carry out sample inventorying, processing, and EIA analysis on fecal samples collected for the sexual maturation and ART projects.

***Collaborating institutions:*** Over the past decade, CREW has established strong relationships with zoological institutions housing polar bears throughout North America. These zoos, as well as the Polar Bear SSP, are highly supportive of CREW's endeavors (see SSP letter of support in Supporting Documents). Approved research and biomaterials requests already are in place at the vast majority of these institutions. Veterinary staff invites CREW scientists to attend anesthetic procedures for semen collections and/or fertility assessments on a regular basis.

### **Dissemination of Results**

For bears involved in the semen collection studies, the institution that houses the bear, the institution that owns the bear, and the SSP will receive a reproductive summary within two weeks of the procedure. Reports for bears monitored as part of the sexual maturity initiative will be generated twice per year for each bear and shared with the housing institution and the SSP. Progress on pregnancy diagnostics will be shared with the SSP regularly. All findings may drive institutional transfers and breeding recommendations. At the conclusion of each of the activities, an update and summary of the findings will be distributed to the Polar Bear SSP, the Polar Bear Research Council, and all institutions housing polar bears. Additionally, it is expected that at least one publication will result for each of the proposed activities, so we expect to submit three scientific manuscripts for publication in Open Access peer-reviewed journals (at least three of these will be submitted prior to the completion of the three-year funding period) so that the findings will be available broadly and at no cost. We frequently describe CREW's polar bear activities at seminars, invited talks, and lectures to visitors and various groups, which totaled 32,502 individuals in FY 2017. The advancements funded by the proposed



project will be incorporated into these talks frequently. More formal dissemination of results will occur at professional meetings, which are regularly attended by CREW scientists, zoological professionals, and reproductive physiologists. Finally, CREW's projects are described and updated on the zoo's website where its *Signature Projects* are specifically highlighted (<http://cincinnatizoo.org/conservation/crew/>) and in the *CREW ReView*, a CREW member magazine with a circulation of approximately 3,500 households.

### 3. Project Results

Our work plan consists of interrelated activities that will conserve collections and result in improved management of polar bears in zoological institutions. This is in seamless alignment with the IMLS category of Collections Stewardship, reflecting the third goal of the IMLS Strategic Plan: *to practice exemplary stewardship of collections and use technology to facilitate discovery of knowledge*. Our goals are to: collect and cryopreserve semen from male polar bears to preserve vital genetics; resolve the uncertainty around the timing of sexual maturation so that precious years are not wasted prior to pairing bears for mating, and; utilize a novel, non-invasive pregnancy test that will guide animal husbandry protocols and will provide insight into where the reproductive process is failing to focus on improved animal welfare. Successful completion of these project activities will enhance the genetic viability, sustainability, and management of polar bear collections maintained in North American zoos. Semen collection and cryopreservation of males will establish fully the world's first polar bear sperm bank. The creation of a permanent polar bear sperm bank will result in a central resource supporting assisted reproduction endeavors and will ensure that the genetic diversity of polar bears is preserved. Characterizing sexual maturity will establish the hormonal parameters for juvenile polar bears and will allow for improved management of the population through more appropriate breeding recommendations. Bears may be recommended for breeding at younger ages, which may allow a female to produce more litters in her lifetime. The ability to diagnose pregnancy will improve collection management, animal welfare, and will provide insight into reproductive failure.

#### Tangible Products

The proposed project activities will result in tangible products that can be broadly utilized. The creation of a polar bear sperm bank will be useful to all zoological institutions that have reproductively viable females, but no males with which to pair them. Currently there are five females, roughly 35% of all reproductively viable females in the U.S. population, without a male for breeding, so AI provides their only chance at having cubs. Of course, the best tangible product of this project would be polar bear cubs that result from AI using semen collected in these initiatives. Monitoring sexual maturation will provide novel information regarding the timing of sexual maturation in this species and will drive breeding recommendations for juveniles in the population. As a direct result of the activities proposed in this project, a novel diagnostic pregnancy test will be created that will allow zoological staff the ability to diagnose pregnancy accurately and non-invasively so that they can manage their bears accordingly.

#### Long-term Sustainability

The entire purpose of the project is centered on long-term sustainability of polar bear populations in zoo collections across North America. Information gained from this project will be disseminated to the polar bear SSP and to all institutions housing polar bears and it will be used to improve management practices of polar bears in captivity. Semen samples collected will be among the first of this species stored anywhere in the world. Long-term sustainability of the project itself at CREW is ensured by the identification of polar bears as one of CREW's four *Signature* projects, which are priority areas of long-term commitment where we recognize our impact will be significant (see Organizational Profile and Letter of Commitment). Results of this project undoubtedly will provide preliminary data for future studies and collaborations. CREW's Polar Bear *Signature Project* has been funded by AZA's Conservation Grants Fund (grants in 2008 and 2014 totaling \$42,852) and numerous private donations (\$606,803 from 2010-2017). At the conclusion of this IMLS Museums for America grant, if awarded, other funding sources, such as AZA's Conservation Grants Fund, Morris Animal Foundation, and private donations will be pursued to continue support for the conservation of this species.

