National Leadership Grants for Museums

Sample Application MG-249011-OMS-21

Cincinnati Zoo and Botanical Garden

Amount awarded by IMLS:	$877,678
Amount of cost share:	$948,533

The project description can be viewed in the IMLS Awarded Grants Search:
https://www.imls.gov/grants/awarded/mg-249011-oms-21

Attached are the following components excerpted from the original application.

- Narrative
- Schedule of Completion

When preparing an application for the next deadline be sure to follow the instructions in the most recent Notice of Funding Opportunity for the grant program and project category (if applicable) to which you are applying.

Narrative

1. PROJECT JUSTIFICATION

Field-wide need, problem and challenges our project will address

Three of the five extant rhinoceros species are critically endangered, one is vulnerable, and the other near threatened with extinction and decreasing in numbers.[1] Although conservation efforts succeeded in growing rhino populations in India and Africa since the 1990s, poaching in Africa rapidly escalated over the last decade causing worldwide rhino numbers to decline for the past two years.[2] Several rhino sub-species have gone extinct in the wild in recent times including the Northern white rhino (*Ceratotherium simum cottoni*)[3] and the Western black rhino (*Diceros bicornis longipes*)[4]. The Bornean Sumatran rhino (*Dicerorhinus sumatrensis harrisoni*) is likely to follow since the last one in Malaysia died recently and very few are thought to exist in Kalimantan. Even the "stronghold" of Sumatran rhinos on Sumatra (*Dicerorhinus sumatrensis sumatrensis*) is precariously hanging on to its existence with a fragmented population estimated at no more than 80 individuals.[2] In fact, the plight of the Sumatran rhino is a stark reminder of the importance assurance populations managed *ex situ* play in wildlife conservation. Although conservationists condemned the effort to breed Sumatran rhinos in human care 25 years ago,[5] it is now upheld as the only path to saving the species from extinction.[6] The strategy is viable largely because of the scientific research of the Cincinnati Zoo's Center for Conservation and Research of Endangered Wildlife (CREW) that turned the failing breeding program into a success, and beacon of hope for the species.[7]

Today, America's zoos provide safe havens for three of the five rhino species, and they are managed by the Association of Zoos & Aquariums (AZA) Rhinoceros Taxon Advisory Group (TAG). Thirty-two AZA-accredited zoos in North America maintain black rhinos,[8] 20 care for greater one-horned (GOH) rhinos,[9] and 47 manage white rhinos.[10] In total, there are ~365 individual rhinos cared for in our accredited living museums, and most are accessible for public viewing by our guests throughout the year. AZA-accredited zoos strive to inspire and educate visitors about wildlife conservation and biological diversity, but they also know certain animals more effectively attract guest attention. Several studies have generated predictable data supporting the theory of visitor bias towards charismatic animals; specifically, visitors exhibit more interest in mammals than in birds, reptiles, amphibians, fish or invertebrates.[11] Furthermore, interest increases with size of animal, and more attention is focused on flagship species when mixed species exhibits are tested. The rhinoceros is a large, charismatic, mega-vertebrate typically displayed as a flagship species and often sought out by zoo guests. A 2015 survey of 36 locations/exhibits at the Cincinnati Zoo & Botanical Garden revealed that the Rhino Reserve was the sixth most highly visited (Attachment 3). It is also well documented in various forms on prehistoric cave paintings and petroglyphs that human fascination with rhinos has a long history.

Regardless of their popularity and public appeal, our zoo rhino populations are far from self-sustaining. Despite our nation's success leading the world in breeding Sumatran rhinos, the species has been phased out of zoos due to insurmountable international politics, with the last calf sent to Indonesia from the Cincinnati Zoo in 2015. Of the three rhino species still in our living collections, only one of the Species Survival Plans® (SSP) is currently green (≥50 individuals in at least three different zoos and on track to sustaining ≥90% of its genetic diversity for the next 100 years), whereas the other two are yellow (not projected to maintain ≥90% of its genetic diversity for the next 100 years).[12] Furthermore, wild rhino imports have been essential to maintaining these *ex situ* populations, but tighter regulations, the poaching crisis, and illegal horn trade have made importations both more challenging and less palatable in recent years. **Therefore, it is critical that we employ a bold approach to address the primary scientific challenges facing rhino management *ex situ* to ensure a long, and healthy (both physically and psychologically) existence for these species in our zoos. Only then, can we provide that safety net from extinction while inspiring our guests with one of the world's most compelling taxa.**

Fortunately, the Rhino TAG has been one of the most progressive animal programs in the AZA, and together with the International Rhino Foundation, developed a visionary strategy by forming the Rhinoceros Research Council (RRC) to guide *ex situ* rhino management based on sound science. The RRC is made up of the nation's leading rhino researchers from multiple disciplines. By 1994, the first Rhino Research Masterplan was drafted highlighting the primary scientific challenges to successfully maintaining rhinos in our zoos, and it has been updated every five years since. The most recent Masterplan completed in 2019 (Attachment 4)[13]

covers a suite of research needs but describes the most pressing subjects under four broad pillars: 1) Impact of and control over body condition/weight, 2) Iron overload in browsing rhinos, 3) Understanding/addressing early and late stage reproductive dysfunction, and 4) Investigating behavioral and environmental factors that affect rhino wellbeing. Acquiring funding to address these research needs is challenging, though there has been some success in specific areas thanks to previous awards from the Institute of Museum and Library Services, International Rhino Foundation, and Morris Animal Foundation. However, the priorities identified above are almost certainly inter-related, and studying one of them in isolation will fall short of producing the results that could be achieved by tackling all of them together in a multidisciplinary, integrated approach. For example, body condition/physical fitness can profoundly impact reproduction, wellbeing, and iron load. **We propose a visionary approach by forming the American Institute of Rhinoceros Science (AIRS) – a model for saving species with science *ex situ*.** AIRS is led by a coalition of RRC advisors and is founded on the partnership between CREW (the nation's premier rhino research program), and The Wilds (one of the most successful rhino breeding and management facilities). The goal of this esteemed group is to take a multi-disciplinary approach to address the priority RRC research pillars. **Throughout the project and beyond, we will broadly disseminate our findings in the form of management recommendations for monitoring and controlling physical condition, iron overload, and reproductive dysfunction, while improving rhino wellbeing.**

How the museum field will benefit and the inclusion of the beneficiaries in the planning

Our nation's zoos know how critical it is to meet guest expectations by providing access to key wildlife species visitors expect to experience while enjoying our facilities. As described above, the rhinoceros is an iconic species that fits the criteria for those receiving the most attention from guests,[11] and current records indicate that 74 AZA-accredited facilities in North America care for at least one rhino species. Therefore, it is essential that we develop sustainable populations of these magnificent animals in our zoos to ensure their long-term access by our communities and associated role as conservation ambassadors, and to serve as population strongholds in the face of dire threats to wild populations. **Let the Sumatran rhino be a lesson to us all in demonstrating the necessity of *ex situ* environments in which endangered species can thrive.** Solid, collaborative, multidisciplinary science is key in understanding the research priorities identified by the RRC. Animal managers are hungry for science-based recommendations and eager to incorporate new suggestions into husbandry protocols for the benefit of individuals and species. Additionally, the public increasingly demands we provide the animals in our zoos with the highest quality of care and utmost respect for their wellbeing. This study will provide substance that is needed to back our claims of doing exactly that.

In addition to benefitting our nation's living museums and the communities that visit them, AIRS will be simultaneously training next generation wildlife scientists to broaden their perspectives while working collaboratively with large networks. The AIRS team purposely encompasses scientists at all stages of their careers. Our study plan relies, in part, on a diverse, inclusive array of interns (high school students, college undergraduates and/or graduates), graduate students and post-doctoral researchers seeking unique experience in wildlife research. Additionally, The Wilds has a proven track record of engaging high school teens from Appalachia, OH,[14] and the Cincinnati Zoo is revered for its Zoo Academy,[15] which offers select teens in the Cincinnati Public School System the opportunity to finish their high school years at the zoo. The diverse students in both programs will have opportunities to learn about AIRS' work and perhaps even witness rhino research in action for an unparalleled experience.

Because our project is based on the Rhino Research Masterplan which was developed by a cohort of 31 rhino experts, including all Rhino SSP coordinators and the Rhino TAG Chair, and representing 11 rhino holding facilities, many of the beneficiaries of our project have already been involved in this program's planning by identifying the research priorities in the 2019 publication.[13] As AIRS' success will rely heavily on the population of rhinos managed at the Wilds, the Project Director and The Wilds' team established buy-in and shared passion for the AIRS concept before it was launched. **The Wilds provides the unique opportunity to study a large, successful population of rhinos cared for under two very different management strategies, thereby allowing each rhino to serve as its own control.** As the discussion evolved, additional experts critical to the success of such a visionary undertaking were engaged. Many of these project Co-PIs represent facilities with additional rhino populations that will bolster the program, including those at Disney's Animal Kingdom, White Oak Conservation, and even Kruger National Park, South Africa, which will be key in

providing wild rhino reference data. In addition to solidifying the commitment of key founders and establishing team leaders for each research pillar, co-advisors, consultants, Rhino SSP Coordinators and the Rhino TAG Chair have all been involved in planning discussions.

Key to AIRS success is transparency and team participation. Early in the planning, the inaugural AIRS members developed an MOU (Attachment 5) and agreed to criteria for co-authorship on scientific publications (Attachment 6). AIRS will expand upon the experience and progress achieved by two previous successful IMLS grants awarded to CREW in 2008 and 2014 that significantly advanced our understanding of rhinoceros reproductive physiology,[16–18] the development of rhino AI,[19] and sperm sorting/banking.[16,20,21] Studies under the reproductive pillar of AIRS focus primarily on understanding and correcting reproductive dysfunction in rhinos with a secondary objective of developing reproductive techniques that complement a recently awarded IMLS grant to Omaha's Henry Doorly Zoo & Aquarium focused on managing rhinos by AI using cryopreserved, sex-sorted sperm. Similarly, studies under the AIRS physical fitness pillar will complement (but not duplicate) a colleague's proposal to validate body condition scores for large mammals.

We are confident that we have canvassed the rhino scientific and management communities, have heeded their suggestions/advice, and avoided redundancy within the research community in developing this program, the results of which will benefit all zoos with rhinos and beyond. **This program will set a precedent for saving species *ex situ* with science, a model with value for everyone managing a zoo species at risk in the wild.**

How the project addresses the NLG goals and aligns with the project category

***Broad impact*:** Results from this program could have significant, direct impact on the management of all ~365 rhinos and their caretakers in North America. Furthermore, our work will help ensure that the guests who visit the 74 facilities with rhinos, which numbered over 75 million in 2019, will be able to experience thriving rhinos far into the future. Finally, we envision our program as a model to be replicated for any species maintained *ex situ* and threatened in the wild. Therefore, the impact of AIRS could be far-reaching on several levels.

***In-depth Knowledge*:** Because AIRS is founded on a document produced and priorities agreed upon by a cohort of 31 rhino experts from around the world, AIRS represents the strongest possible knowledge base for a rhino science institute focused on the advancement of *ex situ* rhino populations. Furthermore, the AIRS coalition is comprised of 17 leading rhino scientists from varied facilities and disciplines that bring diverse expertise, opinions, and experiences to the team. With this outstanding network of collaborators and the transparent approach we are implementing both internally and externally, we have positioned ourselves to be as informed as possible in the planning of our endeavor.

Innovative Approach: **The establishment of AIRS is in and of itself a transformative approach to improving the lives of animals in our zoos; in short, the whole is greater than the sum of its parts when it comes to solving wildlife scientific challenges**. This innovative culture permeates the program and its pillars that include novel ideas (e.g., measuring body iron levels in rhino horn; sorting sperm by sex based on protein expression), custom equipment (rhino accelerometers; rhino iSperm), specialized techniques (deuterated water estimation of adiposity; magnetic sperm sorting) and new biomarkers (vitamin D, prolactin, osteocalcin, glycated hemoglobin) to be tested.

Collaborative Process: The collaborative process in developing AIRS with colleagues and beneficiaries was described in detail above and is supported by the MOU and co-authorship agreements (Attachments 5 & 6) that underline our sincerity and commitment. Additionally, the consensus of all researchers to maintain a centralized database is proof of our team approach and transparency with each other and our beneficiaries.

Shared Results: One of AIRS' primary goals is to serve as a role model in forming an institute of excellence led by a coalition of experts that advances the science necessary to ensure the long-term health, well-being and sustainability of a taxon threatened in the wild. This model will be applicable to any number of species for which *ex situ* populations are essential. Furthermore, within each pillar, we are developing techniques, equipment and new tests, all of which will not only be accessible to the community but will be recommended for use by the community. Our goal is to provide affordable, feasible, science-based recommendations that can be employed by all facilities with rhinos. Our program, and results thereof, will boost our nation's confidence that zoos are committed to saving species while providing the highest standard of care. All studies will be published in open access online scientific journals for easy access by anyone.

AIRS aligns perfectly with the **Collections Stewardship and Access** category. AIRS is addressing the four broad research priority pillars essential to the success of long-term, *ex situ* rhino management. AIRS comprises a strong coalition of rhino experts and their network of colleagues, all of whom have recruited additional participants to ensure we base the program on the best collective knowledge and information available. Our goals are to generate scientific data under all four pillars that drive rhino management recommendations, provide new tools for community use, offer improved standardized procedures for consistency across the nation, and establish validated, informative tests that clinicians at all AZA facilities can use to monitor their rhinos' health and wellbeing. Through these efforts, we will help ensure *ex situ* rhino populations thrive in the future, serving as back up populations to wild counterparts while inspiring and teaching our communities about the wonders of wildlife and the need to protect it.

2. PROJECT WORK PLAN

Project Activities and Evaluation: Specific, hypothesis driven studies have been designed under each of the four research pillars (below) that will answer questions, expand our knowledge base, and guide animal care recommendations to improve the lives of, and future for, rhinos *ex situ*. The success of AIRS will be based on the number of new revelations, hypotheses supported/not supported, questions answered, deeper scientific understanding of the topics, and ultimately, science-based recommendations for rhino care and management.

Project Risks and Mitigation: Potential risks to the program include small sample sizes for some studies or sample groups (e.g., thin rhinos), failed biomarker test validations, rhino management changes that disrupt or confound ongoing studies, animal illness and mortalities, and inadequate biological material for desired testing. These risks will be mitigated by: 1) including our vast network of rhino facilities that participated in our previous rhino IMLS grants plus those in South Africa, 2) including a list of potential biomarkers (Attachment 7) from which to choose knowing that not all will be validated, 3) maintaining close contact with collaborating animal managers so that we are aware of disruptions that could impact study results, and 4) prioritizing tests across pillars to ensure adequate samples for top priorities (Attachment 8). The AIRS team is very cognizant of the level of risk we are taking with each study and have ensured a balance of low-risk and higher-risk studies are proposed (Attachment 9). Admittedly this project is ambitious, but the team is large, experienced, committed, highly skilled, and up to the challenge. Furthermore, we already have support committed by numerous rhino holding facilities (Attachment 10).

Who will Plan/Implement/Manage Project and other Partners: This project will be led by a team of 17 expert rhino scientists consisting of the seven founding members and numerous co-investigators and consultants, all of whom have already been involved in study design, and will play an active role in implementation. Each research pillar has one or two designated Team Leaders (Physical Fitness: Mandi Schook and Michele Miller; IOD: Terri Roth; Reproduction: Parker Pennington; Wellbeing: Elizabeth Freeman and Lara Metrione) who are responsible for overseeing that pillar's activities and played a leading role in developing the pillar studies. The AIRS core team of Co-PIs and Co-Investigators will work cohesively and in concert with animal care staff at participating facilities. Because The Wilds has such a robust, reproductive rhino population and is the facility earmarked for some of the most intensive sampling, biomarker validations and innovative technology testing, a full-time project site Operations Manager will be stationed there to manage the project and interns/graduate students receiving scientific training under the project umbrella. As CREW and The Wilds are ~3 hours driving distance, the Project Director and Operations Manager plan to meet monthly in person to review the program's progress and challenges, and work as a team with the rhinos, equipment, laboratory assays and Wild's animal care team. Additional facilities will include those at which AIRS team members work plus others we recruit as the studies expand in Years 2 and 3 of the program. Several graduate students will be engaged, will travel to study sites to facilitate project implementation and data collection, conduct data analysis and interpretation, and help prepare publications. Partners will be engaged in studies requiring special expertise, material access, equipment and/or statistical analyses. Responsibilities of all key personnel are described further in budget justifications and letters of commitment from external key staff have been received (Attachment 11).

When and in What Sequence Will Activities Occur: Work under all pillars will commence on the starting date of the grant. For some studies, initial work will be conducted on a sub-set of rhinos to validate/test methodologies before expanding to include additional facilities. The first 6-8 months will involve study

preparation, partner facility correspondence, graduate student/intern recruitment and assay validations. The end of Year 1 and Year 2 will be the most labor intensive in sample and data collection. In Year 3, sample collections will wind down, assays will be completed and data analyses/manuscripts will become the focus. See Schedule of Completion for details.

Time, Financial, Personnel and other Resources Needed to Carry out the Project: All seven Co-PIs have already committed substantial time and will commit much more to AIRS when financial support is secured (Attachment 11). The Co-PIs are already established rhino researchers, in their own right, but see this team effort as a unique opportunity for advancing our scientific understanding necessary to save rhinos *ex situ*. Co-Investigators and consultants will commit varied levels of time and effort to pieces of the program. Resources needed include salaries, stipends, housing, travel expenses, lab/veterinary supplies, consultant fees, biomarker assays, small equipment/equipment development, commercial lab fees, conference stipends and publication costs. Details are provided in the budget justification.

Tracking Progress Toward Achieving Intended Results: Overall program progress will be evaluated quarterly during virtual conference calls that include the entire AIRS team and additional co-investigators and consultants as appropriate. The prior quarter's activities will be reviewed and matched to the schedule of completion to ensure the program and individual studies stay on track. Grant activities and measures of progress will include facility recruitment/permissions, sample collections/acquisitions, assay validations, sample testing, data generated, preliminary findings, presentation at conferences, abstracts and manuscripts published and presentations to rhino stakeholders regarding results and recommendations. Additionally, challenges, disruptions and changes to original plans will be discussed as a group during the quarterly calls, and the team will collectively decide best next steps to mitigate program disruption. Each pillar's sub-group will meet/communicate more frequently as needed to review activities/progress within studies specific to each pillar.

How and With Whom Will you Share Your Project Results: Project results will be shared continuously within the AZA Rhino community via presentations at AZA conferences, Rhino TAG meetings, Rhino Keeper meetings/newsletters and updates within the Rhino Research Council. Our goal is to provide specific management recommendations to all rhino holding facilities and to incorporate them into an updated version of the AZA Rhino Husbandry Manual which is electronic specifically for this reason. Additionally, our studies will be shared with the scientific community via multiple peer-reviewed publications that advance rhino science while serving as a model for other wildlife species. When appropriate and of public interest, study results will be shared in the form of press releases for broad general distribution to our communities locally and/or nationally. Our centralized database will be available to researchers long after the grant ends to maximize project productivity and benefits.

Specific Research Activities Under Each Pillar

Much of the initial research proposed will first be conducted on the ~15 white rhinos at The Wilds that are managed in a semi-free ranging manner for half the year and a traditional barn/paddock style the other half of the year. This population will be key in providing paired comparisons while serving as a testing ground for new technologies, assay/methodology validations and the generation of preliminary data. Controls for confounding factors (e.g., season) will be included. Pilot studies at The Wilds will be expanded to include additional rhino species, individuals and institutions (Attachment 10) with varied management strategies. We understand that participation levels will vary across facilities and have drafted participation tiers that detail what is involved so zoos can choose the tier that best fits their program and rhinos (Attachment 8). The complete list of biomarkers and biological samples targeted for each pillar can be found in Attachment 7 with the goal of generating data on many, but not all, of the listed biomarkers as validations may fail for some of those initially tested.

PHYSICAL FITNESS PILLAR

The two objectives under this pillar are to 1) identify measures of health related to physical fitness and 2) examine factors in *ex situ* managed African rhinos related to physical fitness.

Question 1: *Which measures of health are most closely related to physical fitness in rhinos?* **Overarching Hypothesis:** Several serum and morphometric markers related to physical fitness in mammals can be used to understand the range of physical fitness in rhinos managed in human care. **Testable hypothesis:** Serum

markers and morphometric measures of adiposity, metabolism, and inflammation applied in other species will correlate with physical fitness as related to 1) adiposity and 2) activity levels in rhinos. **Theoretical Framing:** In human and animal models, obesity and sedentary lifestyles are linked to infertility, metabolic disruption, inflammation and iron overload;[22] all conditions afflict one or more species of rhino in managed care.[23–26] Abundant food availability and decreased need to forage in managed environments may contribute to decreased physical fitness, but documented health conditions have not been investigated in relation to physical fitness in any species of rhino. To understand the role of physical fitness in health conditions in rhinos, we will assess measures of health in relation to fitness. **Relevance to Practice**: While subjective body condition scoring tools exist for rhinos, these have not been scientifically validated. Thus, methods for assessing health in relation to physical fitness in rhino species are not currently available. Development of a practical tool would be invaluable for tracking health and wellbeing for rhinos in managed care. **Research Methods:** Banked serum will be used to validate commercial assays for physiologic measures of health, metabolism, energy balance and inflammation (see Attachment 7 for potential measures; many already validated for ≥1 rhino species). Serum (monthly) and morphometric measurements (quarterly) will be collected during winter and summer months over one year from rhinos (n=80 white and n=40 black) at participating facilities along with demographic and environmental information collected via survey (see methods for Question 2). Potential markers will be evaluated for variability correlated to 1) body fat, determined using a deuterated water technique employed previously in large zoo animals (n=35 rhinos/species),[27] and 2) changes in resting heart rate and activity levels measured over time using wearable accelerometer and heart rate technologies validated prior to study implementation. Wild rhino serum samples (~20 samples/species) currently stored in South Africa may be analyzed in Year 3 to obtain comparative values for biomarkers of greatest interest.

Question 2: *Which factors in the managed environment significantly contribute to decreased physical fitness?* **Theoretical Framing:** At least one species of rhino in managed care has decreased insulin sensitivity and increased inflammation compared to wild counterparts,[26] suggesting factors related to the managed environment contribute to health risk. However, we do not understand which factors may contribute to decreased physical fitness as an underlying risk factor for health conditions such as iron overload and infertility. **Relevance to Practice:** Understanding how management factors vary across populations in relation to physical fitness will provide direct, actionable results that lead to tools for addressing health and reproductive conditions and recommendations for best care. **Testable Hypothesis:** Factors such as behavior, space use, diet, and general health will correlate with measures of physical fitness and health conditions in rhinos. **Methods:** Using mixed model analysis and the same population cohort and sample collection period as for Question 1, we will test the relative contribution of aspects of behavior (related to activity, space use and rest; see wellbeing pillar), diet composition (nutrient analysis and survey data), and foot health to variability in measures of physical fitness (see Q1), and health conditions such as iron storage and infertility (see IOD and reproduction pillar for measures). Animal demographic data (age, sex, wild caught, etc.), will be extracted from studbooks, and surveys will be sent to participating institutions to record animal foot health, and diet composition across seasons (time on pasture, browse availability, training and enrichment foods, and supplements).

IRON OVERLOAD DISORDER PILLAR

The two objectives under the IOD pillar are to: 1) identify environmental conditions/individual characteristics that exacerbate IOD, and 2) test a novel method for monitoring IOD.

Question 1: *Can we identify environmental factors/individual characteristics associated with higher liver iron loads? Is IOD truly induced by zoo conditions?* **Testable Hypotheses**: H1: Certain environmental and/or individual factors are associated with higher liver iron loads in black rhinos. H2: Iron is positively correlated with other liver mineral concentrations. H3: Wild black rhino tissues contain more iron than wild white rhino tissues. **Theoretical Framing**: For years, scientists have claimed IOD in rhinos develops only *ex situ*, implying that zoo conditions are inappropriate for browsing rhinos and cause disease. This early conclusion was based on one heavily confounded study described in minimal detail.[28] More recently, scientists have suggested that IOD is an evolutionary adaptation that helps black rhinos survive in their natural habitat.[26,29,30] Wild black rhinos have higher serum iron concentrations than white rhinos, and in some locations, concentrations are

similar to those reported in zoo managed rhinos.[29,31,32] Therefore, it is plausible that under certain conditions, wild black rhinos also store iron in organ tissues. Low iron diets already are recommended for all black rhinos in zoos.[33] However, liver iron values vary significantly among individuals[32,34] indicating environmental conditions (e.g., location, climate, diet, years at given zoo, physical condition, lack of parasites, etc.) and/or individual characteristics (e.g., wild caught, captive born, physical fitness, reproductive success, generation, etc.) can exacerbate IOD. It is also possible that tissue iron loading is associated with other minerals since mineral interactions are complex, interactive and often rely on similar physiological pathways and protein regulators for absorption in the gut and systemic trafficking.[35] **Relevance for Current Practice**: Zoos are harshly criticized for improperly feeding rhinos that store excessive iron, but IOD may be driven by inherent physiological processes influenced by environmental factors and not solely due to dietary differences *ex situ*. Identifying those factors associated with higher rhino body iron loads could guide management recommendations to reduce IOD severity. **Research Methods**: A recent analysis of 45 rhino livers, 37 of which are from black rhinos, provides the foundation for this study, but additional samples will be needed to bolster the data set for statistical power when correlating environmental and individual factors (including biomarkers in other pillars). Furthermore, additional GOH and white rhino samples are required to determine true value ranges for species comparisons. Finally, we plan to analyze liver tissues by histopathology and/or mineral analysis that have already been collected from wild black and white African rhinos plus additional samples collected during the grant (~10 samples/species) for wild species and *ex situ* comparisons. Samples representing all three rhino species (n=55) will be sent to Michigan State University (MSU) for mineral content analysis by ICP-MS. Wildlife MSU pathologist Dr. Dalen Agnew has offered his consultancy to ensure consistency in techniques and valid comparisons between populations for any histopathology work included and can perform this assistance via teleconference with South African scientists.

Question 2: *Is there a reliable, non-invasive way for clinicians to monitor iron load in rhinos?* **Testable Hypotheses**: H1: Black rhino horns contain more iron than white rhino horns. H2: Iron content of black rhino horn reflects liver iron load. **Theoretical Framing**: Methods for accurately monitoring IOD progression in rhinos have been elusive. Standard serum biomarkers are yielding unsatisfactory results,[30,36] and liver biopsies are difficult and risky to obtain. The scientific literature indicates that iron content in keratin (fingernails, toenails, hair) reflects body iron levels.[37,38] Like hair and nails, rhino horn is made of keratin and should accumulate iron accordingly. **Relevance for Current Practice**: If an accurate, reliable biomarker for iron load could be identified in a biological sample easily collected, clinicians would finally have the tool they need for monitoring IOD in rhinos and evaluating impacts of management changes based on our recommendations. **Research Methods**: Core rhino horn samples unexposed to environmental contamination and surface rhino horn will be obtained from white (n=10) and black (n=10) rhinos ~1 inch from the base, dissolved, and analyzed by ICP-MS to quantify iron content and compare sample types. Since white rhinos have much lower body iron stores, differences should be apparent if horn has potential as an iron load indicator. If data are promising, 20 black rhino horns matched to liver tissue samples will be analyzed to validate the correlation of horn to body iron concentrations and determine sensitivity and accuracy. Finally, if supported by initial results, surface horn from rhinos in the physical fitness pillar will be analyzed and values compared to biomarkers of health/fitness and wellbeing. Permissions to sample many horns are already in hand (Attachment 10).

REPRODUCTIVE DYSFUNCTION PILLAR

The two main objectives under this pillar are to: 1) understand the etiology and drivers of aberrant reproductive cyclicity (anovulation and acyclicity) and 2) improve sperm quality and availability for desired ART activities.

Question 1: *What factors (management and physiological) are related to reproductive dysfunction in rhinos?* **Testable Hypotheses**: H1: Aberrant cyclicity will be associated with management strategies, physical condition, and/or wellbeing measures in white rhinos. H2: Differences in targeted serum biomarkers (estrogen, prolactin, Vitamin D) will distinguish rhinos with irregular cycles while shedding light on dysfunctional mechanism. **Theoretical Framing**: Aberrations of cyclicity in white rhinos are well documented,[23,39–41] but specific etiologies and drivers are unknown. Further complicating diagnoses are discrepancies in events that manifest in similarly irregular outcomes. For example, anovulation can result from the failure of dominant follicles to ovulate, hemorrhagic anovulatory follicle (HAF) formation, and/or lack of follicle

development.[17,40,41] Elevated prolactin is associated with similar reproductive dysfunction in elephants,[42–45] but has not been explored in rhinos. Vitamin D plays a vital role in many reproductive processes,[46–48] and deficiencies are associated with reduced fertility and uterine leiomyomas,[49–51] a common reproductive pathology in rhinos.[52] Rhino fecal estrogen metabolite monitoring has been uninformative,[17] but with appropriate assays, serum estrogen could provide insight into white rhino follicular dynamics. **Relevance for Current Practice**: Reproductive dysfunctions are common among female white rhinos *ex situ*, rendering few females prolific and many genetically unrepresented. Currently, rectal ultrasonography is required for confirming ovulation and discerning some aberrant reproductive conditions, but most rhinos are not trained for it. In contrast, many black and white rhinos are trained for voluntary blood collection. If serum hormones can be used to accurately diagnose reproductive dysfunction while providing insight into the physiological mechanism and associations with environmental factors, we will finally be empowered to address reproductive dysfunction at its roots, to ensure both reproductive health and genetic representation of female rhinos. **Research Methods**: Serum samples will be collected weekly and feces, nasal secretions or urine 3x/wk for 1 year from female white rhinos (n=20) and analyzed for progesterone to distinguish regularly cycling, pregnant and irregularly cycling individuals while profiling serum estrogen, prolactin, and vitamin D. An additional 30 female rhinos in the physical fitness/wellbeing pillars will be monitored less intensively with monthly serum samples and 3x/wk fecal samples. Once identified, irregularly cycling females will be trained for serial ultrasound exams to detail ovarian activity, ovulation, HAF formation, etc., and validate the reproductive dysfunction associated with serum hormone profiles. Data will then be compared between management styles and to those collected under the well-being and physical fitness pillars to elucidate associations with other factors.

Question 2: *Can sperm processing and preservation be improved and standardized to better serve the rhino community?* **Testable Hypotheses**: H1: Sperm survival post-thaw can be improved by modifying current protocols. H2: New scientific technologies will make possible field-friendly, affordable standardized sperm evaluations and sperm sorting. **Theoretical Framing:** Sperm collection and preservation is well established in male rhinos.[53–55] Thanks to previous IMLS grants to CREW, colleagues at many zoos are trained to perform such procedures, artificial insemination with thawed semen is successful in GOH rhinos,[19] and rhino sperm can be sorted by sex.[20] The value of sex-sorted semen is high, while the feasibility of acquiring it is low, requiring expensive equipment that reduces sperm longevity.[56,57] Recently, there have been reports of affordable, alternative methods for sorting sperm via ligand binding[58] or magnetic nanoparticles.[59,60] A proven animal protein-free medium for rhino sperm[61] may facilitate both sorting and longevity. Furthermore, a field friendly computer assisted sperm analyzer (the iSperm) currently used for livestock[62–64] may be adaptable for rhinos, thereby standardizing evaluations across the many facilities now performing these procedures. **Relevance to Current Practice**: With numerous institutions now trained to collect and cryopreserve rhino sperm, and samples regularly being shared among institutions/technicians, standardization of assessments is key to comparing data and managing banked samples on a national level. Additionally, a current IMLS-funded project is focused on integrating successful sex-sorted sperm AI into rhino management. Once achieved, the demand for sex-sorted sperm will skyrocket and cannot be met with current technologies and resource restrictions. Therefore, a fast, field-friendly, inexpensive method for sorting sperm will complement the achievements of our colleagues and help meet the community's need for large quantities of sorted rhino sperm. Furthermore, because timing AI near ovulation is challenging in rhinos, modified processing protocols and/or supplements that improve post-thaw longevity of sorted sperm will be valuable for increasing pregnancy success. **Research Methods**: Semen will be collected from the male rhinos at the Wilds up to twice yearly and at additional facilities as available (n=5 males/yr). Samples will be used to test methods for improving post-thaw longevity (OptiXcell, +/- potentially beneficial additives), assessing new sperm-sorting techniques (sperm membrane proteins, nanoparticles, ligand binding) and for developing iSperm parameters (Attachment 12).

WELLBEING PILLAR

Question 1: *How do behavioral and physical markers of wellbeing correlate with each other and differ across socio-environmental conditions for white and black rhinoceros?* **Overarching Hypothesis:** Socio-environmental factors impact rhinoceros wellbeing and can be assessed using behavioral (frequency/duration

of behaviors, ratings of temperament) and physiological (heart rate, biomarkers) measures. **Testable Hypotheses**: H1: Species-appropriate behaviors (e.g. mud wallowing, sleep and activity budgets, social interactions) and increased locomotion are positively correlated with social group size and demographics, greater and more diverse spatial access, diversity of food type and presentation, increased grazing or browsing opportunities, milder climates, frequency of training and enrichment, and degree of choice, all of which are positively associated with lower adiposity, better foot health, regular cyclicity, and healthy physiological values. H2: Ratings of temperament correlate with behavior, socio-environmental conditions, and physiological measures of wellbeing. **Theoretical Framing**: There are many possible indicators of wellbeing within five domains: nutrition/feeding, physical health, environment, behavior, and mental state.[65] Some indicators for physical health are validated[23,66–70] and wild rhino behavior can be referenced.[71–75] However, a systematic evaluation of *ex situ* rhino behavior as it relates to the other domains of wellbeing is needed. Some studies evaluated associations between cyclicity, reproductive success, or mortality and social behavior or certain characteristics of the zoo environment,[25,69,76–79] but a comprehensive understanding of the relationships among a greater diversity of behaviors, a more comprehensive suite of environmental variables, and a panel of measurable physiological parameters associated with wellbeing is needed. **Relevance for Current Practice**: Evaluation of animal wellbeing is a requirement of all AZA facilities, and the public is increasingly attuned to animal wellbeing. It is essential to validate indicators used to assess rhinoceros wellbeing and to understand which variables affect wellbeing outcomes. **Research Methods**: Accelerometers and heart rate monitors to distinguish locomotion from resting activity and sleeping[80] will be validated on a sub-set of rhinos through simultaneous live observations.[81] Thereafter, accelerometers, live, and video observations will be used in combination to record white (n=40) and black (n=40) rhino behavioral frequencies and durations. Rhino temperament will be assessed using keeper ratings and novel object tests.[82] Physiological measures collected for research questions throughout this proposal will be used for this question, and blood cortisol:DHEA,[83] oxidized guanosine,[84] and urinary 6-sulfatoxymelatonin[85] will be validated. A survey will be used to collect data on social and physical environments, husbandry methods, and rhino history.

Question 2: *What role(s) do semiochemicals play in rhino social interactions and reproduction?* **Overarching Hypothesis**: Rhinos use chemical communication to maintain social relations and advertise reproductive status to conspecifics. **Testable Hypotheses**: Chemical cues can be used to manipulate reproductive cycles and sociality. **Theoretical Framing**: Research into *in situ* rhino olfactory communication has recently begun. African rhinos are believed to rely upon auditory and olfactory communication because of their poor eyesight.[86] Communal middens[87,88] and spreading feces upon defecation[89] advertises territoriality, age, familiarity, and reproductive state. Chemical signals in feces are an effective form of communication because they are an honest indicator of the status of the sender and persist a long time in the environment.[86,90] Volatile organic compounds (VOCs) from white rhino feces vary with sex, age class, and status of the male and female reproductive state.[87] Interest of wild white rhinos[86] and black rhinos[90] in feces differs based upon the sex, age class, and familiarity/identity of the defecator (sender). **Relevance for Current Practice**: We will build upon the knowledge base generated from studies on free-ranging[90] and *ex situ*[91] rhinos to investigate the efficacy of chemical stimuli in managing populations. We predict that chemical communication is a promising tool for managing rhino behavior in herds, during translocations, and to promote reproductive success.[86,90] For instance, feces from conspecifics (novel or familiar cohabitants) could facilitate male-male social interactions, and stimulate cyclicity and/or reproductive behaviors in females. **Research Methods**: Females identified in Question 1 of the reproduction pillar as experiencing dysfunction will be targeted for this study. They will be divided into two groups and presented feces from either novel or cohabitating breeding age males. The rate and frequency of their behavioral responses will be compared. Impact of the continued presentation of feces over several weeks on the physiology of the rhino (e.g. fecal progestagen metabolites) also will be assessed. A secondary study will focus on male bachelor groups, if any during Question 1 of the well-being pillar are identified to have behavioral challenges (e.g. aggression). Males in bachelor groups will be presented feces

from cohabitating and novel adult bulls and their behavioral and physiological responses (e.g. fecal androgen and glucocorticoid metabolites) will be measured.

Data Generated in all Pillars: Briefly, AIRS will collect physiological, behavioral, categorical, continuous and survey data. All data will exist in a centralized database using the Labfolder software program and made available to AIRS participants for inter-pillar integration, meta-data analyses and interpretation. The database will be developed and managed by one AIRS member who will ensure information is easily downloadable into commonly used statistical programs and will prepare the information for public access. Analyses will include general linear mixed effects models, ANOVA, ANCOVA and Pearson's correlation coefficients. A consulting statistician with established working relationships with AIRS members will be employed for some analyses. Data interpretations and conclusions will be fully vetted by the entire AIRS team to be sure alternative interpretations and confounding factors are considered prior to publication/presentation. (More data details are provided in the digital product form.)

3. PROJECT RESULTS

Project intended results – **With this project, we will demonstrate the tremendous impact a multidisciplinary, multi-institutional scientific approach can have in overcoming challenges to establishing and maintaining thriving populations of species *ex situ* in our living museums.** We will achieve this goal through science-based recommendations derived from our research that are reasonable, feasible, and affordable and will help the zoo community improve rhinoceros well-being, physical fitness, reproduction, and health. We will significantly expand the scientific knowledge of caring for rhinos *ex situ* while building an extensive meta-database of rhino physiological biomarkers and behavior for use in studies that commence long after the grant end date. Our findings will demonstrate to guests and the public at large the extent to which we will go to provide the highest quality of life for endangered species that may someday survive only in our care. Ultimately, we will improve the vitality of *ex situ* rhino populations.

Changes to intended audience – Our science will be convincing and translated to lay language for ease of understanding by the public, our guests, and animal care givers. Our recommendations will be affordable and feasible to ensure they are readily embraced by the rhino, science, and veterinary communities eager to improve rhino care and management. In addition to providing the "what", we will provide the "where" and "who" so rhino caretakers will know where to go for tests and/or whom to contact for advice/assistance. Zoo staff will experience deeper satisfaction in the level of rhino care they provide, and the benefits to rhinos will be enjoyed by zoo audiences in the form of healthier individuals displaying natural behaviors, more offspring, and greater longevity.

Tools and findings adaptable to others and widely disseminated - The under-current of our research strategy is to develop procedures, tools, tests, and applications that are user and field friendly, inexpensive, and easily acquired. The iSperm, protein/magnetic based sperm-sorting technique, rhino horn iron sampling, and rhino accelerometer are all examples of making labor/expertise-intensive methods of acquiring essential biological information easier so that others can readily adopt and utilize them. Our hope is that our collaborating institutions will be touting their utility even before the studies are complete. Discussions among colleagues in addition to presentations at rhino meetings, zoo meetings, scientific meetings and in publications will broadly disseminate the information and introduce the tools/tests we have developed and validated.

Sustainability – The sustainability of our project's results is highly likely because we will be providing the rhino community with actions they can take to avoid/minimize problems and improve rhino care and sustainability**. Our project is giving the zoo community exactly what it is asking for in terms of research that will improve rhino management *ex situ*.** Our results and recommendations also will be incorporated into the Rhino TAG Husbandry Manual and will be part of the discussions when the TAG and SSPs make their breeding and transfer plan recommendations. Several AIRS team members are on the Rhino TAG and SSP Steering Committees and well integrated into the management of zoo rhinos. Finally, the scientific findings will be published in open-access, peer-reviewed journals which will be publicly accessible indefinitely.

	September 1, 2021 - August 31, 2022	Sept-Nov	Dec-Feb	Mar-May	June-Aug
General		Q1	Q2	Q3	Q4
	Establish Operations Manager at the Wilds				
	Develop structure for centralized database				
	Sample collections at the Wilds				
	Interview Interns				
	Intern training at The Wilds				
	Pre-study statistician consult				
IOD Pillar					
	Acquire permissions and rhino horn samples				
	Acquire permissions and rhino liver tissue samples				
	Rhino horn mineral analysis species comparison				
Repro Pillar					
	Rhino sample collections				
	Vitamin D, estrogen and Prolactin assay validations				
	Fecal sample processing/analyses				
	Semen collections				
	Semen studies isperm, sorting, longevity				
Phys Fit Pillar		Q1	Q2	Q3	Q4
	Interview/hire MS student				
	Acquire permissions and draft survey				
	Assay validations				
	Ship supplies to participating facilities				
	Data/sample collections				
Wellbeing Pillar					
	Interview/hire MS student				
	Order supplies and coordinate with zoos for testing				
	Establish/validate behav. data collection protocols				
	Recruit summer interns				
	Arrange with/ship supplies to facilities for study 1				
	Collect data for study 1				

	September 1, 2022 - August 31, 2023	Sept-Nov	Dec-Feb	Mar-May	June-Aug
General		Q1	Q2	Q3	Q4
	Enter data into centralized database				
	Sample collections at the Wilds				
	Interview Interns				
	Intern training at The Wilds				
	Rhino Tag meeting AZA mid-year				
IOD Pillar					
	Acquire permissions and black rhino horn/liver				
	ICP-MS testing for black rhino liver/horn tissues				
	Manuscript preparation/submission				
Repro Pillar					
	Rhino sample collections				
	Preliminary Vit D and Prolactin data generated				
	Fecal/nasal progesterone annual profiles produced				
	Semen studies isperm, sorting, longevity				
	Serum E and P early profiles				
	Manuscript preparation/submission				
	Rhino ultrasound training/data collection				
	Semen collections				
Phys Fit Pillar		Q1	Q2	Q3	Q4
	Data/sample collections				
	Ship samples to lab				
	Endocrine and lab analyses of samples				
	Behavior and Survey data collation				
Wellbeing Pillar					
	Collect behavioral data and samples for study 1				
	Behavioral data analysis				
	Arrange with facilities for study 2 olfactory				
	Ship samples to lab				
	Endocrine and lab analyses of samples				

	September 1, 2023 - August 31, 2024	Sept-Nov	Dec-Feb	Mar-May	June-Aug
		Q1	Q2	Q3	Q4
General					
	Enter last of data and prepare for public use				
	Sample collections at the Wilds				
	Intern training at The Wilds				
	Hold AIRS session at AZA annual meeting				
	Final Sessions with Statistician				
IOD Pillar					
	Import rhino liver from Africa/find lab in Africa				
	ICP-MS/histopath analysis of SA liver tissues				
	Analyze factors associated with liver iron/minerals				
	Manuscript preparation/submission				
	Acquire/analyze horn from phys fit rhinos				
Repro Pillar					
	Rhino sample collections				
	Vit D and prolactin profiles completed				
	Rhino semen collections				
	Fecal progesterone profiles complete				
	Manuscripts preparation/submission				
	Rhino ultrasound data collections				
	Final serum E and P profiles				
	Semen studies sorting, longevity completed				
Phys Fit Pillar					
	Endocrine and lab analyses of samples				
	Behavior and Survey data collation				
	Metadata analysis across pillars				
	Manuscripts preparation/submission				
Wellbeing Pillar					
	Complete data analyses for Study 1				
	Manuscripts preparation/submission				
	Initiate Study 2 olfactory				
	Analyze data from Study 2 olfactory				