National Leadership Grants for Museums

Sample Application MG-251614-OMS-22

Mississippi State University

Amount awarded by IMLS:	$749,086
Amount of cost share:	$0

Mississippi State University will lead a multi-site research project to support the collections care and management of at-risk amphibians in immediate danger of extinction. The project team includes 23 partner institutions that will develop and disseminate new tools to facilitate the preservation and sharing of biomaterials from threatened amphibians for their genetic management; train zoo staff and students on biomaterial preservation of reproductive materials; and conduct foundational research on a one-plan approach that links the conservation of populations in the wild and in zoological facilities through biomaterial banking for improved sustainability. Project findings will help reduce the risk of population collapse of the living collections and maximize genetic diversity through storage of key reproductive cells. The project team will share results in peer-reviewed publications, conferences, video tutorials, podcasts, websites, and social media as well as through advanced training workshops.

Attached are the following components excerpted from the original application.

- Narrative
- Schedule of Completion
- Data Management Plan

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.

The Amphibian Conservation and Biobanking Network

"We should preserve every scrap of biodiversity as priceless while we learn to use it and come to understand what it means to humanity" - E.O. Wilson

1. Project Justification

INTRODUCTION: Mississippi State University (MSU), in collaboration with a consortium of 23 partners, representing academia, zoos, private landowners, federal agencies and state wildlife organizations, are requesting a **$749,086** National Leadership Grant (NLG) from IMLS to support an innovative research and outreach program called 'The Amphibian Conservation and Biobanking Network'. Our team proposes a One Plan approach for amphibian collections care and management that will address a critical need by: (1) linking *in-situ* and *ex-situ* populations of threatened amphibians; (2) conducting research on best practices for expanding a National Amphibian Genome Bank for collections management; (3) providing a much-needed suite of curatorial tools and resources for amphibian collections stewardship to bolster long-term sustainability and genetic diversity; and (4) facilitating training of the next generation of living collections care specialists. The Network will begin by establishing the MSU hub and 3 regional nodes in the Midwest (Omaha Henry Doorly Zoo), Southwest (Fort Worth Zoo), and Southeast (North Carolina Zoo); the MSU hub and regional nodes will work at a continental scale to facilitate the Network's collection objectives for ***at-risk amphibians*** that are in immediate danger of extinction. Consortium partners in these efforts include the Association of Zoos and Aquariums (AZA), AZA amphibian taxonomic group (ATAG), Amphibian ARK, Amphibian Survival Alliance, 6 state wildlife and parks departments, Maryland Zoo, Riverbanks Zoo South Carolina, Houston Zoo, Phoenix Zoo, Zoo Atlanta, Detroit Zoo, Cincinnati Zoo, Colorado's Native Aquatic Species Restoration Facility, Ted Turner Enterprises, and U.S. Fish and Wildlife Service. The Network of partners assembled has received the backing and support of the AZA to establish a national germplasm repository for amphibians due to our team's (1) husbandry and scientific capacity; (2) physical capacity; (3) governance and management; (4) national linkages with other zoos, aquariums, universities, governmental agencies, and museums; and (5) highly successful outcomes and outputs from our previous IMLS grants focused on amphibian biobanking and collections stewardship.

The program goal and associated objective(s) of our NLG project: Goal 3 of the IMLS mission is to advance the museum, zoo and aquarium field's ability to identify solutions that address high priority and widespread collections care and conservation issues, and all three objectives within Goal 3 will be addressed by this project as follows:

1. Objective 3.1: We will develop, implement, and disseminate new curatorial tools, resources and services that will facilitate the preservation and sharing of biomaterials from threatened amphibians for their long-term management.
2. Objective 3.2: We will provide training to curators, veterinarians, keeper staff, graduate, undergraduate, and intern students on biomaterial preservation from threatened amphibians to improve collections stewardship.
3. Objective 3.3: We will conduct foundational research on a "One Plan Approach" for linking *ex-situ* amphibian collections and *in-situ* populations through biomaterials banking for improved sustainability and genetic management.

THE CRITICAL NEED AND HOW IT WAS IDENTIFIED: ***The problem*** - Human activities, both directly and indirectly, have caused alterations or loss of habitats across much of the planet, leading experts to describe the current era as the sixth great mass extinction [22, 31, 26]. Paleontological records show we are experiencing a higher rate of extinction than any previous transition between geological eras [31], with a recent report to the United Nations showing that a quarter of all species on the planet face extinction within decades [6]. Particular victims of this sixth mass extinction are amphibians, with nearly two hundred species already extinct and several hundred more in rapid decline [27]. Of the 8,345 amphibian species documented [1], 43% have declining populations in immediate need of conservation [15]. In response to the amphibian extinction crisis, captive assurance colonies have been established by zoos and aquariums for endangered amphibian species around the world. Although captive assurance colonies have been established for many of the threatened amphibian species in North America, most of these programs are not sustainable and are currently unable to maintain >90% of the populations' gene diversity to avoid inbreeding depression. Also, genetically valuable animals are dying without being represented in the captive collections. *Loss of reproductive output in captive collections limits the number of offspring that can be reintroduced to the wild for overall species recovery*. Even though amphibians are the most threatened of all vertebrate taxa, they have the fewest number of managed survival plans (n=6) within AZA institutions, and only two of these meet full sustainability requirements. Notably, there are no newt or salamander SSPs, placing caudates at even greater risk of extinction (49.8%) compared to anurans (31.6%) [15]. The low reproductive output seen in captive amphibians is often attributed to a lack of unknown environmental stimuli for

breeding, which results in: (1) inappropriate reproductive behaviors; (2) no gamete release; and (3) poor fertilization which quickly leads to colony collapse [20]. Hence, there is a ***critical need*** to reverse this declining trend in our living collections if we are to save a portion of our country's unique amphibian diversity. Therefore, assisted reproduction technologies, in concert with cryopreservation of reproductive cells (e.g., sperm), for genetic management needs to be implemented before aging animals are lost, gene diversity is no longer recoverable, and wild populations go extinct [18].

The low reproductive output from amphibian species in living collections can be minimized and even reversed through development of a Genome Resource Bank (GRB) that serves as a biomaterial repository for reproductive cells. GRBs are a specialized form of living collections whereby captive and wild-type isolates, maintained as living cells, can contribute to perpetual replication of organisms. More importantly, these GRBs preserve sperm, eggs, and embryos that can be used to reconstitute/reanimate species while conserving enough of their genetic heterozygosity sufficient to sustain healthy populations. Presently, the technology only exists for cryopreserving the amphibian male genome (sperm cells), due to their smaller size compared to eggs or embryos and the substantial damage from ice crystallization in the freezing process. The primary goal of the MSU National Amphibian Genome Bank (NAGB) will be to help meet the sustainability goals of the AZA amphibian regional collection plan by augmenting the effective population size long after the death of the gamete donors, thus maintaining up to 90% of all existing gene diversity for an extended period (100 years). GRBs are essentially the archive for which amphibian genetic material can be deposited or withdrawn to produce new animals through Assisted Reproductive Technologies like hormone therapy, gamete collection and *in-vitro* fertilization (IVF). While the goal of our research program is to assist captive assurance colonies by decelerating the loss of genetic diversity, prevent extinction and provide animals for reintroduction, a by-product of our studies has been the generation of a wealth of knowledge about the fundamental reproductive biology for rare amphibians. Sadly, very few resources have been directed toward building national GRB collections and we are now at a critical juncture where if we don't act soon to grow capacity for a National Amphibian GRB, much of our country's amphibian heritage will disappear forever. Thus, it is not far-fetched to suggest that cryobanks may be the last, and only, resort for cataloguing and saving threatened amphibian species for potential retrieval in the future. The ***need for this research has been clearly identified*** as a priority by the AZA amphibian Regional Collection Plan (RCP), IUCN amphibian conservation action plan [15], the Amphibian ARK and Amphibian Survival Alliance Plan. Moreover, the assembled team has been in frequent contact with the AZA amphibian community, working closely with them for over 20 years, and this need is repeated often and strongly at national meetings, through list-serves, and is now cited in USFWS species recovery plans.

In September 2021, the IUCN World Conservation Congress, at its session in Marseille, France, called on all members to "enable and support establishment of a global network of biobanks dedicated to the achievement of global species conservation targets and to integrate conservation for a species both *in-situ* and *ex-situ*, and under all conditions of management." The Species Survival Commission of the IUCN is promoting this strategy, coined the **One Plan Approach** [25, 28]. Although first described in 2013 [5], this strategy has not yet been adopted by zoos and aquariums because they are lacking real world examples of its application. Traditionally, *in-situ* (in the wild) and *ex-situ* (in zoological facilities) conservation practices have not been well integrated and would benefit from mutual planning, implementation, monitoring, and assessment, in order to facilitate the adaptive management process. We propose to utilize the National Amphibian Genome Bank to show how GRBs can be used to bridge this divide. Because most amphibian collections do not fully meet the >90% gene diversity over a 100 years threshold for sustainability, they have to regularly collect wild animals every 4-5 years as new founders to reach certain genetic diversity targets. Given the low numbers of wild populations, removing new founders is no longer a sustainable strategy and was abandoned decades ago for most mammalian species. Instead, we propose that sperm could be collected from wild male frogs, toads and salamanders through hormone therapy (as shown from our previous IMLS grants), frozen in the field, and brought back into the captive collection for future *in-vitro* fertilizations to produce new mixed wild:captive offspring. Thus, a One Plan Approach would create a more sustainable and effective management tool for genetic diversity and collections care.

SPECIES TARGETED FOR COLLECTIONS MANAGEMENT: Each regional node of the Network will target 5 individual species, or in one case a group of salamanders. Omaha Henry Doorly Zoo, as the Midwest node, will work with the *Wyoming toad, boreal toad, dusky gopher frog, Panamanian golden frog and blue-spotted salamander*. The Fort Worth Zoo, serving as the Southwest node, will work with the *Houston toad, Chiricahua leopard frog, Puerto Rican crested toad, hellbender and black-spotted newt*. The North Carolina Zoo will target the *gopher frog, coqui, ornate chorus frog, green salamander, and plethodontid salamanders*. The regional nodes are unique among zoos because of their large collection and numbers of the species listed above, providing large sample sizes of animals to work with and bank down germplasm. Most of the species targeted here are listed as Priority I or II species in the ATAG regional collection plan and are in urgent need of biobanking for long-term collections care. In addition, the majority of these species are listed as

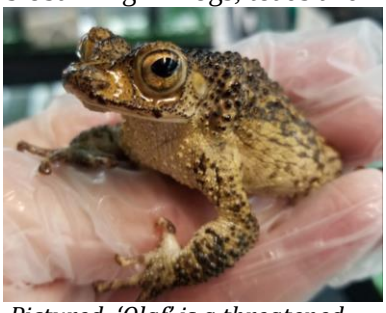
threatened by the IUCN Red-List and are either listed as federally endangered or candidates for listing. Sperm samples from 20-30% of the captive population will be placed into the germplasm repository, which will capture the majority of genetic diversity, while reducing mean kinship from siblings. We will also link the *ex-situ* and *in-situ* populations by traveling to field sites, collecting from wild males (n=5-20 /site), and adding their gametes to the NAGB, thereby bridging the two groups for better management and sustainability of genetic diversity. This is a realistic number of individuals for collection from the wild and will capture a substantial portion of allelic diversity at each location. As the Hub, MSU maintains a research collection of *tiger salamanders, spotted salamanders, plethodontid salamanders, and Fowler's toads*. These animals will be used for refining protocols on novel species if needed.

OUR TARGET GROUP AND HOW THEY WERE INVOLVED IN THE PLANNING: The primary target group or audience for this framework, that will most immediately and positively be affected, are *zoological practitioners*; specifically, administrators, curators, veterinarians, and keepers. There are currently 241 accredited zoos and aquariums within AZA, many of them maintain herpetological collections, employing 1-6 keepers. A conservative estimate of the number of individuals that could be impacted by this project would be 900 employees within AZA (150 institutions x 6 staff) due to the changes resulting from our new paradigm shift for genetic management using an active GRB within living collections. The Amphibian Conservation and Biobanking Network was created as an offshoot program from the AZA regional collection planning process to meet the organization's future genetic management needs. Additional audiences that will be impacted by the Network, but were not necessarily involved with the detailed planning process include: post-doctoral fellows, graduate students, intern students, federal and state agency biologists, private land owners and visitors to the respective zoos and aquariums. There are currently over 23 partner organizations engaged with this project, all of them having participated in developing and supporting the Network.

ULTIMATE BENEFICIARIES OF THE PROJECT: "Beneficiaries" from the creation of this Network and who will most likely be aided in the long-term by our project can be found in our LOGIC MODEL and our table of Audience Members and Benefits, located in the Supporting Documents. They are similar to the "target groups" identified above.

HOW THE MUSEUM FIELD WILL BENEFIT: This innovative NLG project will provide a suite of benefits to the conservation field including: (1) new curatorial tools, resources and services that will facilitate the preservation and sharing of biomaterials from threatened amphibians for their long-term genetic management; (2) training for curators, veterinarians, keeper staff, graduate students, undergraduate students, and interns on biomaterial preservation to improve collections stewardship; (3) hiring of a new diverse group of collections care specialists that will move into the profession; and (4) foundational research that will dramatically expand the work to new amphibian species, demonstrate the economic value of incorporating GRBs into collection plans, and develop a new model of collections sustainability by using the 'One Plan Approach'. For further information we have included a Table and LOGIC MODEL in the Supporting Documents on Audiences served, along with their needs and benefits.

HOW THE PROJECT DIFFERS FROM, COMPLEMENTS, AND BUILDS UPON EXISTING THEORY, SCHOLARSHIP, AND PRACTICE: In the Supporting Documents there are two IMLS summaries on our first 2009 NLG project, titled "*Biomaterial Resource Banking and Assisted Reproduction for endangered North American Frogs and Toads*" and our 2011 NLG project titled "*Development of Assisted Reproductive Technologies for Endangered North American Amphibians: Phase II Salamanders"*. The 2011 NLG grant budget was reduced by IMLS and we were asked to submit the salamander biobanking portion of this project as a follow up proposal, which we did in 2016. These three highly successful NLG projects created a suite of protocols for assisted reproduction and biobanking in frogs, toads and salamanders that were transferrable and easily replicated by zoos around the country. Moreover, these prior awards established the U.S. National Amphibian Genome Bank (NAGB), which contains >1,500 samples of sperm from 13 different amphibian species, many of them threatened with extinction. A number of conservation milestones have been generated from the NAGB including offspring produced using frozen-thawed sperm in seven species, of which three have produced F2 generations; offspring produced from deceased parents; and thousands of F2 generation tadpoles released to the wild. For a list of samples in our repository, please see the Supporting Documents. The tools developed from these earlier projects established the foundation of protocols (i.e., hormone therapy, gamete collection and storage, IVF, cryopreservation strategies) that are no longer in the development stage, but are ready to be executed on a larger scale. These earlier projects also *impacted the attitudes and behavior* of our partners to such a degree that they have created new post-doctoral



Pictured: 'Olaf' is a threatened Puerto Rican crested toad produced through in-vitro fertilization using cryopreserved sperm collected from a wild male

positions at their institutions, hired 5 of the graduate students to work in their research divisions, hired our undergraduate students as summer interns, shared successes with visitors through social media and signage, and are excited to continue the work and establish themselves as permanent regional nodes for the Network. The first three NLG awards significantly impacted a limited number of species, serving as the model organisms, placing them on a more sustainable path within our collections. This proposal is different**,** as we propose to utilize those previously developed protocols **to study** whether we can apply them to a much larger suite of species on a *mass scale using a Network design* for greater effectiveness and outreach. The study proposed here both complements and builds on previous work by dramatically expanding the scale to include new species, measuring the economic value of incorporating GRBs into collection plans, and incorporating new research questions using the 'One Plan Approach' for linking *ex-situ* and *in-situ* populations that will substantially impact how we address sustainability of collections moving forward.

2. Project Work Plan

RESEARCH QUESTIONS, THEORETICAL FRAMING, RELEVANCE TO CURRENT PRACTICE, AND METHODS: The Amphibian Conservation and Biobanking Network of collaborators has three overarching Objectives tied directly to the three components within Goal 3 of the IMLS mission, which include: (1) Foundational research; (2) Collections' stewardship management; and (3) National capacity building.

Objective 1: Foundational Research for Amphibian Collections Management. Our team has identified the 4 research questions below as the most critical for sustainability and genetic management of our nation's amphibian collections and we briefly explain the relevance to current practice, theoretical framework, and methods for each research question under study. As a point of organization, each regional node and hub will address Questions 1-3; however, they will focus their efforts on the species or taxa highlighted above for their region. Question 4 will be addressed as part of the PhD student's dissertation. Summer intern students will assist with research projects the three nodes and Hub. *Our overarching testable hypothesis* is that the expansion of our cryobanking technologies will be positively associated with increased amphibian reproductive output and greater gene diversity, thus changing the relative sustainability index for living collections.

Research Question 1: Will methodologies for sperm collection and cryopreservation, developed in common model amphibians, transfer to important collection species within and between genera or families? Our **Testable Hypothesis** is**:** If protocols for sperm collection and freezing provide on average 35% post-thaw motility in our common amphibian models (e.g., *tiger salamander, fowler toad and leopard frog*), then they should be transferable within genera, and across genera. **Relevance to Current Practice:** There is an urgent need to transfer the products developed under previous IMLS awards obtained in model species to rare species of *conservation concern* and scale up to the next level. As described above, many of the amphibians we have targeted in this proposal are not sustainable in captive collections and require assistance, similar to that provided by our earlier studies. Yet we do not know how well our protocols will translate to different species within the same genus or across genera and families. Having an understanding of this protocol transferability will provide more potential for widespread adoption. **Theoretical Framing**: Our team has developed a suite of assisted breeding and sperm cryopreservation protocols for common amphibian species, and some threatened species, that lay the groundwork for this project [3, 4, 9,10-12, 16, 18, 20]. Milestones from these earlier studies include tens of thousands of tadpoles released into the wild to support recovery programs, offspring produced from frozen sperm, F2 generations produced from those offspring, and the world's largest National Amphibian Genome Bank repository to support conservation efforts. **Methods:** To induce spermiation we utilize gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG) alone, or in combination and evaluate sperm production, percent motility, forward progressive motility, concentration, morphology, and sperm viability over time. For freezing, sperm are mixed with different cryoprotectants, loaded into 0.25 ml straws and subjected to a two-step cryopreservation process over liquid nitrogen vapor. Each straw receives an individual label containing information on species, gender and studbook number along with a barcode linking the sample to a suite of information about the animal (age, weight, birth place, snout vent length captive or wild born, etc.), location and date of collection, and extensive information on sample quality. After cryobanking, sub-samples are thawed for 5 seconds at 45°C in warm water and the same sperm parameters are re-evaluated for post-thaw recovery data. These data are included in the biorepository Freezerworks® software program, which links the samples' specific accession number and barcode label with the data collected.

Research Question 2: Can sperm be collected and gene-banked from recently deceased or diseased animals that are no longer part of the breeding population but are genetically important to preserve? Our **Testable Hypothesis is**: If viable and motile sperm can be extracted from testis of deceased male amphibians, cryopreserved, and used for replication of new individuals, then we should impact the population's genetic structure. **Relevance to Current Practice**: In general, amphibians are short-lived animals and can experience a high death rate in captivity due to stress, nutritional challenges,

and missing environmental stimuli. As a result, genetically important animals will occasionally die without passing along their genes to the next generation. Moreover, several priority species within AZA's regional collection plan suffer from moderate rates of disease in captivity (e.g. chlamydia, cryptosporidium, bacterial and fungal etiologies). These diseased animals are often removed from the breeding population, and occasionally euthanized, to prevent zoonotic spread. Currently, there is no coordinated effort to recover the reproductive potential of these genetically valuable animals upon death, nor are there any easily accessible protocols available for how to extract and freeze testis macerates or where to send samples for processing. **Theoretical Framing:** Male anuran sperm has been obtained from deceased and sacrificed individuals, cryopreserved, and motile or viable sperm observed post-thaw [17]. Moreover, tadpoles have been produced from testis macerates of several frog species, where sperm was frozen from deceased individuals [29,30]. Theoretically, we should be able to transfer this work to salamanders or other anuran species and utilize the genomic material for applied reproductive management and collections sustainability. **Methods**: All experimental procedures for Question 2 will be *opportunistic*, as we cannot predict disease outbreaks or sudden death of animals (although they occur regularly). Male amphibians euthanized due to disease outbreaks will allow us to immediately conduct controlled experimental trials on the impact of different cryoprotectants and different freezing rates on testis macerate, similar to Question 1. Pre-freeze and post-thaw sperm parameters will be collected as described above, which will allow us to compare the two different approaches of using testis macerates vs. spermic urine collected from live animals. Male amphibians found deceased in enclosures will have their testis removed by an attending veterinarian, placed into a sperm preservation media, kept chilled on ice at 0^0C and shipped overnight to one of the regional nodes or Hub for processing. Testis will be macerated, sperm evaluated, and cryopreserved. Testing this work on deceased animals will require us to be extremely adaptive (e.g., different storage/transport temperatures or medias) as sperm may be particularly susceptible to degradation if left too long in the carcass. For impact on genetic structure see section *below on data analysis*.

Research Question 3: Does linking *ex-situ* collections and *in-situ* populations through biobanking improve genetic management and sustainability? Our **Testable Hypothesis is**: If amphibian sperm can be cryopreserved from wild males and used to produce mixed offspring of captive:wild parents, then we should be able to reduce importation of live animals and improve gene structure. **Relevance to Current Practice**: In order to maintain >90% genetic variation over 100 years within AZA's living amphibian collections, programs must regularly collect 5-10 new animals from the wild every few years to introduce new founders. For threatened amphibians with low population numbers this conservation strategy is no longer sustainable for managing both *in-situ* and *ex-situ* populations, nor does it support most zoo conservation missions. Instead, collection and cryopreservation of sperm from wild males and storage in a germplasm repository would: (1) allow the animals to remain in the wild; (2) avoid shipment and quarantine issues that present risk; and (3) extend the lifespan of the genetic material long past living organisms. **Theoretical Framing**: A pilot grant from the AZA conservation endowment and Disney's Conservation fund allowed us to conduct preliminary experiments with the endangered *Puerto Rican crested toad* to show proof of principle for this strategy. This pilot project was highly successful resulting in the production of >40 adult offspring produced from frozen-thawed sperm, with the adults having now matured and reproduced themselves, resulting in the release of >5,000 tadpoles into the wild [4]. Theoretically, we should be able to easily *transfer* and *replicate* this strategy across multiple amphibian species. **Methods**: Each regional node will work on executing field trials to collect wild males from their key species, administer hormones as described previously [4,19], collect sperm and freeze it in the field using a mobile lab set-up, and conduct IVFs with the frozen sperm using captive female. We will utilize optimized protocols determined from Research Question 1 for cryoprotectant media and freezing rates. Five of the species that we will work with have detailed pedigree analysis and studbook information (*Wyoming toads, Puerto Rican crested toads, dusky gopher frogs, Panamanian golden frogs, and Houston toads*); thus, we will be able to describe our influence on the microstructure of the population through heterozygosity analysis, inbreeding potential and contribution of founders to the living population (i.e., gene diversity changes). See the *data analysis section* below for how we evaluate this data.

Research Question 4: What is the economic cost-benefit relationship to collecting and importing cryopreserved sperm from wild male amphibians for collections management, instead of removing animals from the wild? Our **Testable Hypothesis is**: Managing the long-term genetic diversity of amphibian living collections is more affordable and sustainable when combined with a genome resource bank of captive- and wild-type sperm. **Relevance to Current Practice:** Estimates suggest that global capacity for amphibian captive assurance colonies can protect no more than 50 species [2] and substantial costs are associated with these captive insurance populations. In addition to economic costs, amphibian populations may suffer from inbreeding depression, [8], loss of fitness [24] and adaptation to captivity [7]. Although some erosion of genetic diversity is likely, AZA has established a target of maintaining 90% genetic variation for 100 years as their gold standard for species survival plans. This gold standard may be difficult to reach for many

living collections due to the high cost of maintaining populations, while avoiding genetic erosion. The solution to this challenge is to combine live collections with frozen repositories of germplasm and show the economic feasibility of this One Plan approach. **Theoretical Framing**: Two recent studies have attempted to estimate such cost-benefit relationships for including genome resource banks into genetic management plans using the threatened Oregon spotted frog [13] and several Australian anurans [14]. While these two studies have laid the theoretical groundwork, they did not have accurate biobanking costs, were conducted in foreign currency and did not have detailed pedigree analysis completed for the entire population like we will have for the five species targeted here. **Method 4:** To evaluate the potential economic impact of incorporating a genome resource bank into individual species genetic management plans we will use detailed actual costs of captive breeding programs from 5 different species where detailed pedigree analysis is available, specifically the *Houston toad, Wyoming toad, Puerto Rican crested toad, dusky gopher frog and Panamanian golden frog.* We will model several 100-year captive breeding cost-benefit scenarios where the size of the live captive colony is required to maintain different levels of the source population heterozygosity or effective population sizes. Our proposed scenarios will include avoiding inbreeding depression in the short-term, retaining evolutionary potential in perpetuity and retaining single locus genetic diversity. Moreover, costs derived from these five programs can be modeled at different theoretical genetic retention goals to include 90, 95 and 99% heterozygosity and compare (a) live colonies only vs. (b) live colonies + a genome resource bank of frozen sperm. The model could predict different cost-benefit scenarios for using scenario (b) as cryopreserved founder sperm are reintroduced through IVF at various generational intervals. The economic model will contain a suite of variable costs for live animal management related to facilities, equipment and consumables, veterinary expenses, labor costs related to husbandry staff, animal food, utilities, and administration. Likewise, biobanking costs will be calculated to include, start-up costs related to tank purchases and small equipment, curatorial labor, liquid nitrogen, consumables related to IVF, travel, utilities, and administration.

Objective 2: Creating Resources for Amphibian Collections Stewardship. The purpose of Objective 2 is to develop, implement and disseminate new resources, curatorial tools, and services that will facilitate the management, preservation and sharing of biomaterials from threatened amphibians for long-term stewardship. Ultimately, the goal is to translate the science into practice at a national scale. First, this IMLS NLG project would allow for the establishment of a suite of resources at each regional node in the Midwest, Southwest and Southeastern U.S. These resources include cryotanks and related equipment for hosting a genome resource bank, along with subject matter experts trained in managing the resources and conducting the research. Second, development of new curatorial tools for genome resource banking will occur throughout the research program as protocols are refined and finalized for dissemination. The network will utilize a shared website (https://conservationphys.msstate.edu) to host and disseminate many of the resources developed. For example, two videos or white boards per year (6 total) will be produced by the MSU TV station that will be disseminated within the zoological community on topics including: (1) Collecting sperm from frogs and toads; (2) Collecting sperm from salamanders; (3) How to cryopreserve and thaw amphibian sperm; (4) Producing offspring through in-vitro fertilization; (5) Dissection and removal of testis from deceased frogs, toads and salamanders; and (6) Organizing field trips for collection and cryopreservation of amphibian sperm from live wild animals. In addition to our video tutorials, we will produce and host several Podcasts, write 1 Blog per month on news related to milestones and successes; add material to social media including our Facebook, Twitter and Instagram accounts; contribute to publications, reports, and conferences; lead training workshops; and produce signage that will be displayed in the Herpetariums at the 3 regional zoo nodes that potentially reach nearly 3 million visitors per year. Lastly, our consultation services are available to zoos and aquariums in need of help with assisted reproductive technologies for conserving threatened amphibian species.

Objective 3: National Capacity Building for Managing Amphibian Collections. This NLG program will build national capacity at various levels. At the ***individual level***, capacity will be increased by hiring and training the next generation of collections specialists including: 1 post-doctoral fellow at Fort Worth Zoo, 2 research associates at North Carolina Zoo and Omaha Henry Doorly Zoo, 1 PhD student at Mississippi State University, 12 undergraduate intern students and dozens of volunteers between all the institutions. All collaborating partners are committed to long-term positions and placing these individuals within the AZA community upon completion of the project. Having gone through this leadership program, the 16 employed individuals will have gained experience in biomaterial preservation for threatened amphibians to improve collections stewardship. Also at the ***individual level***, capacity building means we will have hopefully changed attitudes and behaviors by imparting knowledge and developing skills in these individuals while maximizing the benefits of participation, networking, ownership and work place exchanges. Changes in knowledge, behaviors and skills will be measured through our formative and summative evaluations described below. At the ***diversity, equity and inclusion level***, all three regional nodes and Hub will make concerted efforts to hire individuals from underrepresented and underserved communities of color. For example, MSU is uniquely staged to assist with training a

more diverse workforce as our student body is nearly 30% minority and the state demographics are nearly 40% minority (https://www.census.gov/quickfacts/MS). We recruit diverse graduate students and undergraduate intern students from within our own student body and nationally from two organizations, Minorities in Ag and Natural Resources (MANNRS) and Annual Biomedical Research Conference for Minority Students (ABRCMS). At the ***institutional level***, capacity will be built at the three regional nodes and Hub improving organizational resources (e.g. regional genome banks with associated equipment and expertise), collections management performance, ability to manage genetic resources more efficiently, as well as better prepare zoological institutions to adapt to change due to animal population or environmental stochasticity. At the ***systemic level***, we believe that the overall policy framework for how the collective organizations within AZA operate and interact for managing 'live' animal populations will be dramatically impacted by incorporating cryopreserved genomic material from both captive and wild populations into the genetic management structure for better sustainability. The network will participate in advocacy initiatives for our 'One Plan Approach', AZA consultations, open dialogue on genetic management, and likely reforms to species survival plans. *Leadership Analysis-* As part of our evaluation plan related to *Objective 3*, it is our intent to follow the professional career of those hired as part of this three year program to understand the impact the mentoring and leadership opportunity had on shaping early-career development for young scientists. We will follow each participant in the program for 5 years following their completion of the program; metrics will be linked with our previous IMLS grant totals for an overall understanding of demographics, change in diversity within institutions, field of occupation, number of majors supported, impacts on professional development, and impact on the institution(s).

TYPE OF DATA WE WILL GATHER AND HOW IT WILL BE ANALYZED? ***Quantitative Data Analysis-*** To date the majority of our studies utilize factorial arrangements with randomized complete block design. Main effect factors in the experiments typically include the cryoprotectant and thawing temperature, which typically results in multiple treatment combinations with 3 or more replicates. *Data collected for research questions 1, 2, and 3* related to sperm cryopreservation experiments will include number of responders to hormone treatments, sperm motility, viability, concentration, and morphology pre- and post-freezing trials. Data for female amphibians targeted in this study include number of animals laying eggs, egg numbers, percent cleavage of embryos, neurulation rate, larvae numbers, metamorphosis rates and animals reintroduced to the wild. Power analysis has been performed using over 10 years of standard deviation data to determine a sample size between 10-20 animals are needed for each treatment group. All data will be tested for normality and homogeneity of variance using the Shapiro-Wilk test. Non-parametric analyses may be necessary when data are not normally distributed. For multiple comparisons of means we primarily use Tukey-Kramer least significant difference, paired Wilcoxon Signed-Rank test, or Student *t*-tests for pairs of means. Analysis of variance and Linear Mixed Effects Models for evaluations over time will be used where appropriate. Data will be expressed as the mean ± SEM and differences will be considered significant at $p<0.05$. For Question 4 we will conduct standard incremental cost-effectiveness ratio (ICER) analysis, which is a form of economic analysis that compares the relative expenditures and outcomes of two or more strategies that perform the same task. The predicted rate of loss of heterozygosity under the different scenarios above will be derived from the relationship between the inbreeding coefficient and heterozygosity. This type of genetic analysis will help determine the residual heterozygosity at each generation. The increase in inbreeding between generations in the captive colony without backcrossing, and the effect of recurrent backcrossing using frozen founder sperm on the rate of inbreeding for the hypothetical populations modelled will be evaluated using an iterative process. ***Collection Stewardship Impact*** –Five species that we will work with have detailed pedigree analysis and studbook information on all the animals; thus, we will be able to describe our influence on the microstructure of the population through heterozygosity analysis, inbreeding potential and contribution of founders to the living population (i.e., gene diversity changes). Pedigree analysis, using the open-source software program PMx (an IMLS-derived product), of the population's studbook data before and after the study will provide an *evaluation* of whether additional individuals contribute new genes into subsequent generations and the partitioning effect of their contribution.

POTENTIAL RISKS TO THE PROJECT: Given the substantial amount of experience from our team and long history of working with many of the targeted species, this project has a high chance for success; however, we acknowledge that no research project is ever problem-free. Potential risks in this project are primarily related to the foundational research questions for *Objective 1*. Specifically, what if protocols developed in our model species do not transfer as well to new targeted species, especially across families? We have observed this on occasion with our work for anurans (frogs and toads). In that event, we will have to conduct additional hormone or sperm freezing trials in order to tweak the hormone dosage, timing or freezing strategies. Another potential problem with Question 1 is there could be challenges with acquiring eggs from female salamanders of new species, such that it is difficult to test the functionality of our gene-banked sperm and determine its applicability to collections care management? If difficulty collecting eggs from some

female salamander species were observed, we would have to adaptively manage our timeframe and focus more effort on the female reproductive strategies, including oocyte maturation and ovulation, utilizing hormone therapy combined with ultrasound analysis. A potential risk for research Question 2 is related to the rapid degradation of sperm in deceased males, which would require adaptive management of the testis extraction protocol and cryopreservation. Research Question 3 has the risk of the team not being able to obtain as many wild males for sperm collection from some *in-situ* populations that we are targeting, given their low numbers and rarity in the wild. Unfortunately, this is the nature of working with threatened and endangered species in the wild or zoos & aquariums. To mitigate potential issues related to sample size, we have targeted enough species combined, which will provide us with significant quantifiable data to make inferences on the successful nature of the program and its national impact.

INDIVIDUALS WHO WILL PLAN, IMPLEMENT, AND MANAGE THE PROJECT: Dr. Carrie Vance will serve as project director for the Amphibian Conservation and Biobanking Network, with Dr. Andy Kouba serving as Co-PI and Curator of Biobanked Amphibian Collections. Both Dr. Vance and Dr. Kouba will oversee the PhD graduate student; who will spend their time on the 4 research questions and will be assisted by several undergraduate intern students. Economist, Dr. Ganesh Karunakaran, will supervise the economic analysis part of the project (Question 4), while human dimensions professor, Dr. Kevin Hunt, will serve as **evaluator** to the project and will assist with the formative and summative evaluations. Diane Barber, Jessi Krebs and Dustin Smith will serve as project supervisors and curator of collections for the three regional nodes in the Southwest, Midwest and Southeast, respectively. The three zoo curators will supervisor the post-doctoral fellow and 2 research associate's projects at each node and will help supervise specimen data entry and database management; intern students will be supervised by the post-doctoral fellow and research associates. Our comprehensive and transparent *training and mentoring plan* for graduate students, undergraduate students, post-doctoral fellows and research associates can be found in the Supporting Documents. The network currently has in place guidelines for our Collection Policy (acquisition, approval, accessioning specimens, field vs. captive amphibians, gifts and donations, permanent loans, purchases), Care of Collections (documentation, accession and catalogue records, specimen database, data labeling, physical map of repository, storage protocols) and Use of Collections (access, ownership, memorandum of understandings established for federally listed species with USFWS, agreements with state conservation agencies). In particular, we have established a 3-way division of stored genomes for the following priorities: (1) a semi-permanent long-term repository for use when a species approaches extinction; (2) a routine management and collection stewardship of living animal collections; and (3) biomaterials for research. We utilize the software **Freezerworks®**, a leading industry standard for managing frozen biorepositories to keep track of all our stored samples. We are linking our collection to the Global Genome Biodiversity Network (GGBN; http://data.ggbn.org) data portal whose goal is to make genomic collections discoverable for research through a networked community of biorepositories and provide trusted and transparent access to genomic samples for users and contributors in a benefit-sharing framework.

TIME, FINANCIAL, PERSONNEL, AND OTHER RESOURCES TO CARRY OUT THE ACTIVITIES: The Amphibian Conservation and Biobanking Network will be a 3-year, **$749,086** project, with detailed information shown in the *Schedule of Completion, Budget, Budget Justification* and *Key Personnel* attachments. With financial support from IMLS we will be able to hire a PhD graduate student at MSU and place research associates and postdoc at each of the three major regional nodes to facilitate the grant objectives; this request to IMLS in support of **personnel** is our greatest financial need to carry out the proposal's activities (personnel costs account for 58% of the total budget direct costs). MSU and the three regional zoo nodes will provide intern students, volunteers and keepers to assist the graduate students and research associates with their respective projects. A mentoring plan is provided in the Supporting Documents. If funded, the grant would also provide some small equipment (e.g. microscopes, pipettes), supplies (hormones, syringes, slides, etc.), and travel (zoos, aquariums and the field), in order to carry out the research. Professors, curators, keepers, and federal and state wildlife biologists will contribute their time, facilities, vehicles, collection animals, and existing equipment to the project. Previously funded IMLS-NLG grants have allowed us to build substantial capacity at MSU for curating the genome resource bank collection, managing a centralized database, and support for the regional nodes.

TRACKING PROGRESS TOWARD ACHIEVING INTENDED RESULTS: The Network's progress will be evaluated quarterly to document its success in meeting its objectives, conformance with proposed timelines and milestones. Key formative evaluation questions will be provided to our zoological partners to collect feedback on the projects impact to collections, zoo curators, zoo keepers, veterinarians, graduate students, research associates, interns and volunteers, using the survey software program 'Qualtrics'. *MSU human dimensions faculty member and partner on the grant, Dr. Kevin Hunt, will serve as the internal evaluator* to ensure that an extensive evaluation is conducted on the project. Dr. Hunt, as evaluator, will provide objective formative evaluation services for this National Leadership Program. His services related to tracking progress will include: monitoring, fidelity checks and audits, assistance with development

of annual progress reports, and establishment of the formative evaluation system. The main purpose of the formative evaluation system will be to collect, analyze, and disseminate data over the course of the project to help the project partners and staff to stay "on track" in implementing project activities and to promote ongoing project improvement. To this end, formative evaluation data will be shared with project partners and staff on an ongoing basis throughout the project. The network team's formative and summative evaluation survey questionnaires do not meet MSU IRB review qualifications. The entire project team will meet quarterly through Zoom conference calls to discuss the formative evaluation and our team will meet in-person once per year at the AZA Herp Conference, where everyone will receive feedback and reports on progress toward goals and outcomes. The MSU team will meet twice a month to report on progress within the Hub, disseminate findings (particularly digital media output) and refine deliverables as needed.

DISSEMINATION: ***(1) Publications, Conferences, Husbandry Manuals and Reports*:** The network will share its findings in peer-reviewed publications, book chapters, popular press articles (e.g., AZA Connect Magazine and partner Zoo's newsletters), husbandry manuals, and press releases. We will also share our results at conferences such as the AZA annual Herp TAG meetings. ***(2) Video tutorials, Podcasts, Websites and Social Media***: In addition to standard print and conference dissemination, our team will also use a combination of internet portals and social media to distribute our outputs including through a series of: 1) six YouTube Videos on our developed technologies; 2) regular updates on our biobanking network's Twitter, Instagram and Facebook pages; 3) A regularly updated NEWS section within our website; 4) one blog per month on successes; 5) a three-part Podcast series; and 6) two progress report postings/year to AZA listserves. The video tutorials, social media sites, podcast downloads etc. will all be accessible through the MSU Hub website at https://conservationphys.msstate.edu and will have information and links at each node institution's conservation pages (e.g. https://www.fortworthzoo.org/conservation); we will also create a new website specifically for the National Amphibian Genome Bank database and information (nagb.msstate.edu). These platforms will be used to share significant achievements or milestones with visitors, members and peers during the project period and long afterwards. ***(3) Signage at Partner Institutions***: Project partners have all agreed to create signage regarding the project goals at their respective institutions so that the public can learn about the amphibian extinction crisis and how this IMLS-supported project is addressing the problem through collections care research and management. These story lines have the ability to reach millions of visitors per year between all the partners. ***(4) Advanced Training Workshops****:* Our protocols and outputs from this project will be shared with our partners' staff (curators, veterinarians, keepers, etc.) as part of our training outreach when we are visiting each institution working with their animals. In addition, the AZA Amphibian Taxonomic Advisory Group will host an Advanced Training Workshop on Amphibian Reproduction in Year 3 of the program that will be tuition-based (restricted to 20 attendees), similar to previous successful workshops we have hosted.

3. Project Results

INTENDED RESULTS AND HOW THEY ADDRESS THE NEED: Projected Outcomes from this multidisciplinary and national research initiative will be *transformative* to the participating institutions. *First*, the foundational research design of Objective 1 will: (1) create sustainable, multi-disciplinary projects where science helps inform management; (2) build project '*buy-in'* through expansion of the Network to regional nodes that shares leadership and adoption of the program; (3) expand the reproductive technologies to more amphibian species, creating a larger impact on collections conservation; (4) demonstrate how a *One Plan Approach* model can revolutionize genetic management and sustainability of collections; (5) show the economic value of genome banking to assist with adoption; and (6) ultimately increase the number of threatened amphibian species that can be repatriated to the wild to support recovery programs. *Second*, the resources designed as part of Objective 2 will: (1) produce curatorial tools, such as step-by-step videos, manuals, and reports that show how the techniques are *exportable and easily replicated*; (3) establish digital platforms for the wide dissemination and broad acceptance of the resources; and (3) publicize/market the tools and digital platforms across a wide suite of social media, popular press, print, signage at zoos, podcasts and websites. *Lastly*, national capacity addressed in Objective 3 will be built addressing our identified needs and problems by: (1) *hiring new leaders in the field* of curatorial management that will integrate best practices of recruitment and retention; (2) creating unique learning environments within the Network that increase reproductive output of amphibians and strengthen the genetic diversity of taxa within their collections; (3) foster culturally diverse and equitable training and leadership programs that strengthen the skill sets, knowledge and attitudes of our partners; and (4) transform the industry by establishing regional nodes that are sustainable and revolutionize how we approach collections management using a *One Plan Approach*.

KNOWLEDGE, SKILLS, BEHAVIORS, OR ATTITUDES THAT WILL BE CHANGED: As part of this IMLS program, we believe that gains in knowledge, skills, confidence and interest will be generated in different members of our

audience and stakeholders that participate in the research and training programs. Summative evaluation methods will measure these changes within our target audiences and stakeholders using pre- and post-surveys; *we have partnered with Dr. Kevin Hunt, human dimensions faculty member at MSU, to provide this summative evaluation of the project.* As internal evaluator, Dr. Hunt will be responsible for the design, establishment and analysis of the summative evaluation system using the 'Qualtrics' software program. One example of proposed changes within our audiences is professional development of research associates, graduate students and undergraduate students such that 100% of this audience will understand how to develop and implement assisted breeding protocols and cryopreservation techniques for amphibians and will feel confident in serving as future leaders and trainers in this field of collection stewardship. Moreover, they will have acquired skills as trainers themselves and be prepared to sustain the activities beyond our core group.

MODELS, TOOLS, RESEARCH FINDINGS, AND SERVICES RESULTING FROM OUR PROJECT: This project will develop the *first model of its kind* for how to implement a *'One Plan Approach'* for AZA collections management, by actively exchanging genes between captive and wild populations for better sustainability. The One Plan Approach model will provide a road map to AZA on how to integrate conservation for a species collection both inside and outside its natural range, and under all conditions of management, by engaging all responsible parties and all available resources at a national level, from the very start of any species conservation planning initiative. A number of *new tools* will be created including hormone therapy protocols, IVF techniques, sperm freezing procedures, an expanded frozen repository of amphibian germplasm, and a searchable database of genetic material available for use in captive breeding management and collection stewardship. The tools, research findings, services, outputs and outcomes from the Network will be widely disseminated as described above and we refer the reader to this section for more details. In brief, *publications, conferences, husbandry manuals, reports, video tutorials, podcasts, websites, social media, popular press, signage at partner institutions and advanced training workshops* will broadly share the results from this project.

SUSTAINABILITY OF THE PROJECT: Through a sustainability planning process we have developed a number of ways to sustain the project past the conclusion of an IMLS grant award, including the framework of a sustainability plan. There are six principal parts to the sustainability plan, which includes the following. *(1) AZA and USFWS community adoption of the resource as critical to operations:* As part of their mission, both AZA and USFWS are committed to endangered species conservation, invest substantial resources into reintroductions, and annual tadpole/larvae releases are part of nearly every federal recovery program. The MSU National Amphibian Genome Bank collection will be integral to producing these animals for release and maintaining the genetic diversity and sustainability of both AZA and USFWS programs long into the future. *(2) Successful outcomes, leveraging of new resources*: The collective institutions participating in this project will raise awareness of the amphibian crisis and highlight dynamic milestones occurring throughout the program, especially those that produce tangible and exciting results (e.g. first breeding of certain species in captivity and/or release of animals to the wild). These outputs from the team in the past, especially through digital media, have brought in additional funding from foundations, private donors and companies that want to be a part of this tangible and dynamic conservation program for amphibians, thus helping to leverage additional support. *(3) Capacity building and training*: Keepers, veterinarians and curators at our collaborating institutions will be trained on the protocols developed as a result of this research proposal. Our goal is to 'train the trainer' and begin the widespread use of these novel technologies and gene banking strategies for increasing their reproductive output and managing gene diversity long into the future. Creating the regional nodes is a substantial step toward institutionalizing this national program. *(4) New leadership*: The principal and co-investigators will recruit and train new professional scientists (2 research associates, and 1 postdoc located at the nodes, 1 PhD student at the hub, 12 undergraduate intern students at both the nodes and hub) in the field of zoological and keeper/curatorial research. It is anticipated that upon completing their programs they will enter the zoological or aquarium field and continue to contribute towards amphibian conservation and collections stewardship. *(5) Dedicated, permanent positions demonstrating long-term commitment:* Addressing the amphibian extinction crisis and collection stewardship emergency has been a part of our team's long-term goal now for nearly 20 years. As such, the University has permanently funded both Co-PIs salaried positions to provide leadership over the National Amphibian Genome Bank. The MSU facilities are partially supported internally through the organizations operating budget (i.e. not solely dependent on soft money) and will continue long into the future. Moreover, the three nodes have committed to sustaining a rotating research associate trainee or post-doctoral positions at their institutions to continue to work on this project past the termination of the grant. *(6) Diversification of funding*: The network's future funding plan past the grant program includes a suite of different strategies including (a) fee for biobanking service; (b) annual fund campaign; (c) major gifts program; (d) new donor acquisition for endowments; (e) internet donation site; (f) state, federal and NGO grant programs; and (g) corporate sponsorships that use logos/icons of amphibians. The University has systems in place to assist with all of these activities to create a sustainable funding model.

SCHEDULE OF COMPLETION:	Year 1				Year 2				Year 3			
	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
Project Set-up- Hiring personnel, Set-up of Laboratory Space, Digital output platform												
Graduate student MSU												
Research Associates at NCZ and OHDZ												
Postdoctoral Researcher at FWZ												
Objective 1: *Foundational Research for Collections Management*	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
1. **Expanding methodologies to important collections species across genera and families.**												
Research data collection, methodology enhancement, gamete collection for NAGB												
PhD Student training of Research Associates, implementation at Nodes and satellite sites												
2. **Developing methodologies for deceased or diseased animals.**												
Research data collection, new protocol development, gamete rescue inclusion in NAGB												
Research Associates at Nodes, opportunistic sampling for emergency response												
3. **Linking in-situ and ex-situ populations for import of founder genetics.**												
Research data collection, new procedural development, wild founder lines into NAGB												
Research Associates at field sites, coordination with breeding season caudates & anurans												
4. **Economic cost analysis of the One Plan approach to living collection's sustainability.**												
Economic of collections sustainability w/wo the NAGB: a road map for AZAs One Plan Approach; Qualtrics assessment of change in attitudes for/against												
PhD student Research, Risk-reward economic modeling, modeling of impact and efficiency												
Objective 2: *Creating Resources for Collections Stewardship*	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
Genetic representation - more species and individuals' gametes stored in NAGB for breeding												
Curatorial tools-creating open access information and guidance												
Protocols for gamete collection and storage, shipping, handling and applications of ART												
Imaging database for reproductive assessment, gamete viability, identification												
Video Tutorials (2x year minimum) of procedures in Assisted Reproductive Technologies												
Reference Base of publications, manuals, book chapters												
Informative Economic analysis on NAGB value and use for collections stewardship												
In person training for collaborators during on-site visits or upon request												
Workshops and open discussions associated with ATAG Meetings												
Objective 3: *Building National Capacity for Amphibian Collections Sustainability*	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
Physical Resources – The National Amphibian Genome Bank sperm repository: MSU / Nodes												
Digital Resources - ACB Network Interactive Website – hosted by MSU												
FreezerWorks digital library of genetic lineages for breeding management												
Information and Education: Outreach and change in attitudes of the One Plan Approach												
Services – consultation services provided to Zoos and aquariums for ART procedures												

Data Management Plan

Types of Data to be collected: Describe the type of data (e.g., digital, non-digital), how it will be generated, amounts, purpose or intended use. Describe methods, scope and scale and dates of data collection.

1. **Non-digital primary data**- gametes, eggs, tadpoles, urine, hormone swabs, testes from deceased animals. Samples collected from animals by inducing gamete release with hCG and GnRHa. Tadpoles to test cryopreserved sperm viability will be generated by in-vitro fertilization. Expected Average Numbers: n=15 species, 15 males per species, 5 cryostraws per animal per year x 3 years. Total estimated banked sperm samples on average 3375 cryopreserved sperm samples. Sperm samples are intended for assisted breeding by AZA accredited zoos for sustainable collections management. Physical samples can be shared via dry shippers. Collections during Years 1-3.
2. **Digital Laboratory primary data**: hormone ELISA analysis, cell physiology data parameters and viability data from gamete collections and cryopreservation trials, developmental data of tadpoles using Gosner scale, animal background and physiology data. Collections during Years 1-3.
3. **Digital Imaging primary data**: Female ultrasound images, video and still capture of sperm parameters through microscope. Video and still images of spermatophores and collection in the wild. Collections during Years 1-3.
4. **Digital record of Genome Resource Bank using FreezerWorks® software**. Data input includes individual animal identification, physiology parameters (sex, age, species, genetic lines), sample date collected, freezing medium, sperm parameters. Collections during Years 1-3.
5. **Digital Tutorials:** Video tutorials (n=6) of procedures and methodology will be generated for information and training purposes of collections care personnel in techniques for preserving gametes from their own collections. Tutorials will be generated in Years 1-3.
6. **Digital Evaluation primary data**- Surveys on the change in knowledge, behaviors or attitudes of our audiences (zoo staff, students, research associates, etc.) will be accomplished through the platform Qualtrics by an internal evaluator, following trainings and as a summative evaluation. Collections during Year 3.
7. **Digital Economic Analysis primary data**- Data collection from zoos involved in project, in real time, for cost of collections care, gamete acquisition and cryostorage, time and effort analysis of IVF breeding using frozen sperm, success of incorporating new genetic lines into known populations, total survivability per cost investment and total outputs. Collections during Years 2-3.

Data format: For scientific data to be readily accessible and usable it is critical to use appropriate community-recognized standard and machine readable formats.

1. **Non-digital primary data**: Sperm samples are cryopreserved and stored in liquid nitrogen, Physical samples of testes macerates obtained from deceased animals are stored cold and then cryopreserved similarly. Hormone samples (swabs or urine) are stored in ultra-low freezer at -80^{o}C.
2. **Digital Laboratory primary data**: Hormone ELISA analysis, Cell physiology data of cryopreserved gametes from research activity is stored as tables and spreadsheet data using MS Excel .XLSX or .TXT files for universal ease of use & accessibility.
3. **Digital Imaging primary data**: Digitally stored as pictures in TIFF or PNG or JPEG files or as designated output from instrumentation for Ultrasound Imaging.
4. **Digital record of Genome Resource Bank using FreezerWorks® software** information will be available from MSU to all nodes using the multi-institute license for FreezerWorks® held by MSU (2021). General species, numbers of animals and know pedigree information will be provide in spreadsheet table format on the website,
5. **Digital Tutorials:** Video tutorials of procedures and methodology will be in easy access video formats for smartphone use on site or in situ. Links will be provided through the website or social media links for ease.
6. **Digital Evaluation data:** Changes in knowledge, behavior, skills and attitudes will be quantified through a survey instrument using Qualtrics Software and stored above as described.

7. **Digital Economic data:** Economic data will be stored in spreadsheets and modeling of impact analysis included in reports and as supplemental data in publications. It may also be used by AZA as a meta-analysis for the One Plan approach to living collections care and sustainability. Results will also be published in open access peer reviewed manuscripts and are accessible through the research team upon request.

Long-term Data storage, preservation and dissemination: Data must be stored in a safe environment with adequate measures taken for its long-term preservation.

1. **Data will be shared** in the open access website to be developed for the ACBN under the Mississippi State umbrella. Back up data will be stored with the Node institutions.
2. Dr. Carrie Vance and Dr. Andrew Kouba will curate **long-term storage** of the physical samples of sperm in the Amphibian Genome Resource Bank. Physical samples will be collected and initially stored in cryotanks at each node (Fort Worth Zoo, North Carolina Zoo, Omaha Zoo, and at MSU), and then coordinated and distributed for secondary back up to MSU. Other physical samples will be stored at Mississippi State University at -80^{o}C in the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology. The facilities management includes temperature alarms monitored 24/7 by staff, faculty and students. back-up -80^{o}C Freezers are on standby in the case of power-outages, freezer malfunction and pandemics.
3. All these files will also be stored on the University's IT managed Google cloud storage-drives for faculty owned by Dr. Carrie Vance and Dr. Andrew Kouba at Mississippi State University. These have no cost and unlimited capacity with weekly backups.
4. Digitized images, spectral files and economic modeling data and files will be stored in the records at the university with back up records at the corresponding zoos and with members of the Amphibian Taxon Advisory Group. Additional long term storage of digital data may be stored on the Ag-Data Commons site under the account curated by Dr. Carrie Vance. This no-cost account is managed by the USDA.
5. Total needed capacity for digital file storage is < 1TB. Proportions of data capacity are approximately: 60% digitized imaging of still and video records of sperm physiology and dynamics, ultrasound imaging library, tutorials of procedures for gametes, collection, cryopreservation, IVFs, and assessments; 20% processed data models and economic analysis, 10% compilations of tables, figures and reports, 20% Freezer works software digital library for the FreezerWorks® biobank record.

Review of Data Management Plan

1. The Data Management Plan produced will be reviewed annually by the Network senior personnel, specifically Drs. Carrie Vance, Andrew Kouba and Allison Julien, and Node Curators Diane Barber, Dustin Smith and Jessi Krebs. All of these key personnel are highly invested in the long-term success of this network and will be using the resources generated regularly for collections management at each of their own institutions. The review will address ease of use of the resources and information available; assessment of depletions and surpluses in the physical sample collection of the biobank; needs in species contributions, numbers of animals and genetic lineages represented and to address any improved protocols for better quality samples to replace earlier versions. There will also be a discussion of capacity within the MSU biobank and the distributions across biobanks held at the nodes to manage needed duplication of the libraries for biosecurity.
2. We will also need to review and update the FreezerWorks® software program for compatibility as digital platforms evolve overtime. Our current version of the biobanking software was acquired in 2021 and replaced the 12 year old version from 2009; as the software does not evolve at an excessive rate it we are confident that it will remain valid long-term. Additionally, the FreezerWorks® software is used by many Human and Agricultural gamete freezing entities in private industry.