



National Leadership Grants - Museums

Sample Application MG-260105-OMS-26

Project Category: Collections Stewardship

Project Type: Research

Mississippi State University

Amount awarded by IMLS: \$679,325

Amount of cost share: \$0

Mississippi State University, in collaboration with a consortium of partners representing zoological parks, aquariums, private landowners, academia, federal agencies, and state wildlife, will lead a research and outreach program to address amphibian population sustainability. The project team will expand support nationally for assisted reproduction, cryobanking, and decision support technologies, and test an AI-driven tool that integrates data about genomes, husbandry, and cryopreservation to assist in making recommendations for species prioritization. By sharing methods that ensure genetic diversity among captive colonies of threatened amphibian species that could be vulnerable to collapse, the project will strengthen sustainability indices for living collections.

Attached are the following components excerpted from the original application.

- Narrative
- Schedule of Completion
- Digital Product Plan
- Public Access 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 to which you are applying.

The Amphibian Conservation and Biobanking Network: Building a National Safeguard for Amphibians.

"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 an expanded consortium of partners, representing zoological parks, aquariums, private landowners, academia, federal agencies and state wildlife organizations, is requesting a \$749,325 National Leadership Grant (NLG) from IMLS to support the innovative research and outreach program called **'The Amphibian Conservation and Biobanking Network: Building a National Safeguard for Amphibians'**. Building on the remarkable success of our earlier efforts, supported by IMLS, that significantly advanced amphibian conservation science and collections care and management, this new initiative will expand the One Plan approach to address the next set of critical needs by: (1) linking in-situ and ex-situ populations of threatened amphibians; (2) conducting research on best practices for expanding the 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; (4) integrating an AI-driven 'Genomic Rescue Advisor' platform that synthesizes conservation status, genomic data, cryopreservation feasibility and husbandry practicality to generate collections-ready prioritization and sampling guidance; and (5) training the next generation of living collections care specialists.

Here we propose to expand the Network by establishing two new regional nodes: Detroit Zoo (Great Lakes region) and Denver Zoo (Rocky Mountain region). The Omaha Henry Doorly Zoo and Fort Worth Zoo continue as a funded Midwest and Southwest node, respectively. Notably, North Carolina Zoo and Fort Worth Zoo, leveraging the success and foundation established through earlier IMLS awards, have independently secured funding to continue their amphibian biobanking programs in parallel. This achievement demonstrates the catalytic impact of IMLS investments and allows the Network to expand its geographic reach and taxonomic coverage while maintaining strong collaborative partnerships with these original institutions. **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 include the Association of Zoos and Aquariums (AZA), AZA amphibian taxonomic advisory group (ATAG), Amphibian ARK, Amphibian Survival Alliance, U.S. Fish and Wildlife Service, five State Wildlife and Parks departments, Maryland Zoo, Riverbanks Zoo South Carolina, Houston Zoo, Phoenix Zoo, Zoo Atlanta, Cincinnati Zoo, Colorado's Native Aquatic Species Restoration Facility, Ted Turner Enterprises, and now the Detroit and Denver Zoos as newly integrated nodes. The Network of partners assembled continues to have the backing and support of the AZA to establish a national germplasm repository for amphibians due to our team's (1) expertise and scientific capacity; (2) physical capacity; (3) governance and management; (4) national linkages with other museums, zoos, aquariums, universities, and state and federal governmental agencies; and (5) highly successful outcomes and outputs from our previous IMLS grants focused on amphibian biobanking and living museum's 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 fields' 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 long-term collections care management.
 2. Objective 3.2: We will conduct foundational research on a "One Plan Approach" for linking *ex-situ* amphibian collections and *in-situ* populations through biomaterials banking for long term sustainability and genetic management
 3. Objective 3.3: We will provide training forums and workshops to curators, veterinarians, keeper staff, managers, graduate, undergraduate, and intern students on Assisted Reproduction Technologies (ART) and biomaterial preservation from threatened amphibians to improve collections stewardship and explore current and emerging issues that inform the field.
- Moreover, IMLS has identified artificial intelligence as a key opportunity area for museums and libraries, emphasizing AI literacy, responsible use, and alignment with federal AI education priorities ([EO 14277](#)). Our project responds directly to this emphasis by developing and integrating an AI-enabled 'Genomic Rescue Advisor' as a core piece of collections infrastructure, using AI not as a novelty but as a practical tool to help museums and zoos make data-informed decisions about which amphibian species and lineages to preserve, interpret, and share with the public.

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 [29, 35]. Paleontological records show we are experiencing a higher rate of extinction than any previous transition between geological eras [34, 38], with a recent report to the United Nations showing that a quarter of all species on the planet face extinction within decades [7]. Particular victims of this sixth mass extinction are amphibians, with nearly two hundred species already extinct and several hundred more in rapid decline [1, 28]. Of the 9006 amphibian species documented [1], >41% have declining populations in immediate need of conservation [18]. In response to the amphibian extinction crisis, captive assurance colonies have been established by zoos and aquariums for endangered amphibian species around the world [2]. 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 [9, 10]. Also, genetically valuable animals are dying without being represented in the captive collections [37]. *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 [37]. Notably, there are no newt or salamander SSPs, placing caudates at even greater risk of extinction (>49%) compared to anurans (32%) [18]. 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 [19]; and (3) poor fertilization which quickly leads to colony collapse [26]. 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 need to be implemented before aging animals are lost, gene diversity is unrecoverable, and wild populations go extinct [24].

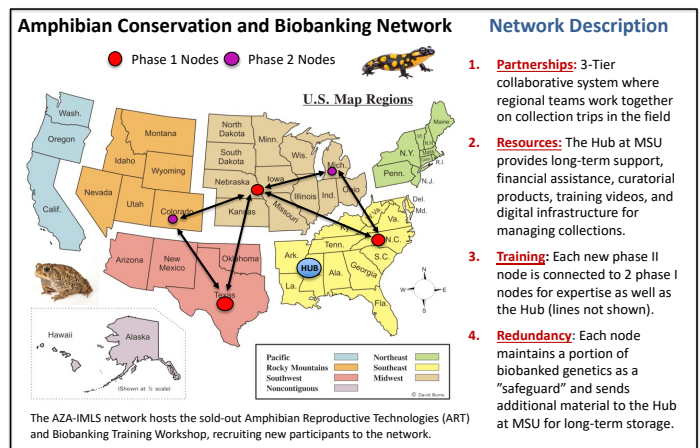
The low reproductive output from amphibian species in living collections can be minimized and even reversed through development of a Genome Resource Bank (GRB) that serves as a biomaterial repository for reproductive cells. GRBs are a specialized form of living collections whereby captive and wild-type 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 [9, 16, 32]. 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 [19]. 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 [18], 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 25 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** [33, 36]. 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 year threshold for sustainability, they have to regularly collect wild animals every 4-5 years as new founders to reach

certain genetic diversity targets [35]. 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 [21, 27, 37]. 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: This project expands the geographic and taxonomic scope of the Network by incorporating regionally significant species at each node. Detroit Zoo, serving as the Great Lakes node, and Omaha Zoo continuing as the Midwest node, will focus on the *Wyoming toad*, *boreal toad*, *dusky gopher frog*, *Panamanian golden frog* and *blue-spotted salamander*. Denver Zoo will target Rocky Mountain region species including *boreal toad*, *northern leopard frog*, *Colorado river toad*, *Amargosa toad* and *Wyoming toad*. Fort Worth Zoo and North Carolina Zoo will continue their work with the *Houston toad*, *Chiricahua leopard frog*, *Puerto Rican crested toad*, *black-spotted newt*, *gopher frog*, *coqui*, *ornate chorus frog*, *green salamander*, and various *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, many 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 ($n=5-20/\text{site}$), 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, hellbenders, and Fowler's toads. These animals will be used for refining protocols on novel species.

OUR TARGET AUDIENCE 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 >240 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 would be 1,200+ employees within AZA (200 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 expanded geographic reach of this project spanning the Great Lakes and Rocky Mountain regions significantly broadens the Network's national impact. 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. Detroit Zoo and Denver Zoo were selected as new nodes based on their staff attending the 2025 IMLS-ATAG amphibian biobanking workshop, demonstrated expertise in amphibian husbandry, regional conservation priorities, institutional capacity, and commitment to long-term program sustainability. Additional audiences that will be impacted by the Network, but were not necessarily involved with the detailed planning process, include: graduate students, intern students, federal and state agency biologists, private landowners and visitors to the respective zoos and aquariums. There are currently dozens of partner organizations engaged with this project, all of them having participated in developing and/or supporting the original 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, government managers, graduate students, undergraduate students, and interns on biomaterial preservation to improve collections stewardship; (3) hiring a new generation of collections care specialists into the profession; and (4) foundational research that will dramatically expand the work to new species, demonstrate the economic value of incorporating GRBs into collection plans [16,17], and develop a new model of collections sustainability by using the ‘One Plan Approach’.

HOW THE PROJECT DIFFERS FROM, COMPLEMENTS, AND BUILDS UPON EXISTING THEORY, SCHOLARSHIP, AND PRACTICE:

Previous 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 now contains >3,000 sperm samples from 27 different amphibian species (triple the amount since initiating the biobank), 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 [27]; offspring produced from deceased parents [19, 20]; and thousands of F2 generation tadpoles released to the wild. The tools developed from these earlier projects established the foundational 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. The Amphibian Conservation and Biobanking Network established three regional nodes (Omaha, Fort Worth, and North Carolina Zoos) that successfully demonstrated the viability and impact of the Network model. These nodes *impacted the attitudes and behavior* of our partners to such a degree that they created new positions at their institutions, hired several of the graduate students to work in their research divisions, hired MSU undergraduate students as summer interns, shared successes with visitors through social media and signage, and are excited to continue the work. The success of Fort Worth Zoo and North Carolina Zoo recently in leveraging their participation to secure independent, additional funding through AZA demonstrates the catalytic impact of IMLS investments and validates the Network model. This project is different and builds strategically upon our foundational work by proposing to: (1) expand geographic coverage by adding Great Lakes (Detroit Zoo) and Rocky Mountain (Denver Zoo) nodes, expanding our national representation; (2) broaden taxonomic scope to include new regionally significant species; (3) scale proven protocols to a much larger suite of species using the validated Network design for greater effectiveness and outreach; (4) advance ‘One Plan’ approach research by incorporating new biogeographic regions and in-situ/ex-situ linkages; (5) evaluate the genetic fitness of cryo-produced offspring; (6) operationalize an AI-enabled ‘Genomic Rescue Advisor’ for collection-ready prioritization and sampling recommendations; and (7) build on proven success by continuing the previous nodes while integrating lessons learned into new node establishment. This proposal both complements and builds on previous work by expanding the national scale and impact, answering new research questions, and maintaining the scientific approach that characterized our earlier successes.



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

2. Project Work Plan

SPECIFIC ACTIVITIES, THEORETICAL FRAMING, RELEVANCE, AND METHODS: The Amphibian Conservation and Biobanking Network has three overarching Objectives tied directly to the three components within Goal 3 of the IMLS mission, which include: (1) development and dissemination of new collections management tools; (2) foundational research; and (3) national capacity building through forums and workshops to inform the field.

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 at the five 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 translating these techniques to many different species within the same genus or across genera and families requires ground truthing and knowing protocol transferability will provide more potential for widespread adoption. Next, 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)[20]. 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 [23]. **Theoretical Framing:** Our team has developed a suite of assisted breeding and sperm cold-storage and cryopreservation protocols for common amphibian species, and some threatened species, that lay the groundwork for this project [3, 4, 6, 8, 11, 24, 26]. 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 spermiogenesis 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 [4, 15]. 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 a sample's specific accession number and barcode label with the data collected. In vitro fertilization uses thawed sperm to fertilize eggs obtained from gravid females determined by ultrasound imaging [13, 14, 30].

Research Question 2: 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, 11, 25], collect sperm and freeze it in the field using a mobile lab set-up, and conduct IVFs with the frozen sperm using captive females. We will utilize optimized protocols determined from Research Question 1 for cryoprotectant media and freezing rates [6,12]. 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 3: Will offspring produced using archived cryopreserved sperm exhibit comparable genetic fitness to offspring produced using fresh sperm, such that they can be responsibly integrated into breeding programs for collections sustainability and species recovery? Our **Testable Hypothesis is:** If offspring generated from cryopreserved sperm show

similar survival, growth, morphology, reproductive performance, and genetic diversity metrics as offspring generated from fresh sperm, then cryopreserved sperm can be used to augment captive and wild populations without compromising population viability or long-term adaptive potential. **Relevance to Current Practice:** Amphibian conservation programs are increasingly interested in using cryopreserved sperm to retain or restore genetic diversity and to support recovery of small or declining populations. However, there is legitimate concern among zoo partners and wildlife agencies that cryo-derived offspring could experience reduced fitness, either through subtle genetic or epigenetic effects of freezing [22, 31], or through inadvertent selection for sperm that survive the cryopreservation process. Without data on relative fitness, managers cannot fully evaluate the risks and benefits of releasing cryo-produced offspring into the wild or using them as breeders in assurance colonies. Establishing that cryo-derived animals have comparable genetic fitness to conventionally produced animals will increase confidence in deploying amphibian genome banks as standard tools in conservation breeding and reintroduction planning. **Theoretical Framing:** Conservation genetics and zoo population management frameworks stress that maintaining genetic diversity and minimizing inadvertent selection in captivity are vital to preserving the evolutionary potential of threatened species. Assisted reproduction and cryopreservation can capture underrepresented lineages, reduce inbreeding, and reconnect gene pools, provided offspring remain competitive and free of latent genetic loads. By testing key indicators of genetic fitness (heterozygosity, allelic richness, inbreeding coefficients, multigenerational performance) and examining epigenetic markers of cryo-stress, this project meets contemporary calls to integrate quantitative fitness metrics into conservation breeding and translocation programs. **Methods:** We will generate paired cohorts of offspring using fresh and cryopreserved sperm from the same males, fertilizing standardized egg batches from females of known origin. Offspring will be reared under identical husbandry, and we will quantify survival to metamorphosis, age and size at metamorphosis, growth rates, morphology, and basic performance traits (e.g., locomotor capacity). At sexual maturity, a subset of individuals from both cohorts will be bred to produce F2 offspring, enabling assessment of reproductive performance and multigenerational viability. Tissue samples from parents, F1, and F2 progeny will be genotyped using microsatellite and/or SNP markers to estimate individual heterozygosity, allelic richness, inbreeding coefficients, and relatedness across treatment groups. Where feasible, we will incorporate assays of DNA integrity or other molecular markers to detect potential cryo-associated damage [22]. These quantitative fitness and genetic data will be linked to specific cryopreservation protocols and stored alongside existing biorepository records, providing managers with evidence-based guidance on when and how cryopreserved sperm can be safely used in species recovery efforts.

Research Question 4: Can an AI-driven decision platform that integrates conservation status, genomic data, husbandry feasibility, and cryopreservation readiness improve how museums and zoos prioritize amphibian species and populations for ex situ collections and germplasm banking? **Our Testable Hypothesis is:** If we build a Genomic Rescue Advisor that systematically ingests conservation assessments, genomic metrics, and husbandry/cryobiology information, then it will generate valuable priority rankings and sampling plans that align closely with expert judgment, while revealing under-recognized amphibian taxa and lineages that warrant urgent biobanking and collections sustainability. **Relevance to Current Practice:** Museums and zoos are increasingly expected to manage both living and cryo-collections as strategic conservation assets, yet decisions about which amphibian species and populations to hold, breed, and bank are often made piecemeal, with limited integration of genomic data or long-term planning tools. Collections staff, SSP coordinators, and Amphibian TAG leaders lack a user-friendly system that brings together threat status, genetic erosion, feasibility of assisted reproduction, and institutional capacity into a single, defensible framework. As a result, scarce tank, rack, and liquid-nitrogen space may not be directed toward the taxa where ex situ intervention and biobanking would have the greatest conservation payoff. Developing a ‘Genomic Rescue Advisor’ as collections infrastructure will help IMLS-supported institutions use data-informed, genomic-grounded criteria to prioritize amphibian accessions, germplasm sampling, and collaborative projects, while generating interpretable outputs that can be shared with institutional collection managers and the public. **Theoretical Framing:** Contemporary conservation genomics and zoo population-management theory emphasize maximizing adaptive potential, retaining evolutionary distinctiveness, and targeting limited resources where genetic rescue and ex situ assurance can meaningfully reduce extinction risk. Decision-support tools in conservation increasingly rely on multi-criteria frameworks that combine extinction risk, genetic status, feasibility, and cost to make transparent tradeoffs. By encoding these principles into a usable platform for amphibian collections planning, this objective aligns with calls to treat genomic and cryo-collections as core museum assets and to integrate a One Plan approach thinking into ex situ program design. **Methods:** We will design the Genomic Rescue Advisor as a modular, web-accessible platform with four components: (1) a curated data layer that aggregates conservation status, literature-derived life-history and threat information, available genomic datasets, and published or inferred cryopreservation/assisted-reproduction feasibility for priority amphibian taxa; (2) an analytics layer that standardizes basic genomic metrics (e.g., diversity and fragmentation indicators) where data exist and uses structured rules to classify species into tiers of cryo-readiness and husbandry feasibility; (3) a prioritization engine that applies an adjustable, weighted scoring scheme (extinction risk, genetic erosion, feasibility, institutional leverage) to generate ranked lists and sampling recommendations at

species and population levels; and (4) a user interface that allows partner institutions and Amphibian TAG representatives to explore rankings, view the key factors driving each score, and export reports for collections committees and planning documents. During the grant period, we will co-develop weighting schemes and validation exercises with TAG and institutional advisors, comparing Advisor outputs to expert consensus and iteratively refining the system. The resulting tool and documentation will be shared with participating museums and zoos, enabling them to incorporate Genomic Rescue Advisor outputs into their collection plans, biobanking strategies, and public interpretation of conservation decision-making.

Objective 2: Expanding 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 U.S. regional node including in the Midwest, Southwest, Southeast, Great Lakes, and Rocky Mountain regions. These resources include biobanks, cryopreservation equipment and related consumables 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; popular press articles; 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 at the regional zoo nodes; potentially reaching nearly 6 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: research associate support at Fort Worth Zoo and Omaha Henry Doorly Zoo, 1 PhD student at Mississippi State University, 7 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 10 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 workplace exchanges. Changes in knowledge, behaviors and skills will be measured through our formative and summative evaluations described below. At the ***institutional level***, capacity will be built at the five 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 five years after completion of the program; metrics will be linked with our previous IMLS grant totals for an overall understanding of demographics, change in hiring 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*** - Most studies use factorial arrangements in a randomized complete block design, with cryoprotectant type and thawing temperature as main factors, producing multiple treatment combinations with at least three replicates. Data collected include male response to hormone treatment, sperm parameters for survival and functionality before and after freezing, as well as female outcomes

such as egg production, embryo cleavage, larval survival, metamorphosis, and reintroduction success. Power analysis from a decade of variance data indicates 10–20 animals per treatment group is sufficient sample size. Data will be tested for normality (Shapiro-Wilk), with nonparametric tests used as needed. Comparisons will rely on Tukey-Kramer, Wilcoxon, or t-tests, and trends over time analyzed by ANOVA or Linear Mixed Models. All data are reported as mean $\pm$ SEM, with significance at $p < 0.05$. **Collection Stewardship Impact:** Five species with detailed pedigree analysis and studbook information allows us 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.

POTENTIAL RISKS TO THE PROJECT: Our team's extensive experience and long history with the targeted species give this project a strong likelihood of success, though we recognize no research effort is without challenges. Most risks are tied to *Objective 1*—specifically, whether protocols developed in our model species will transfer effectively across new species or families. We have occasionally seen such variability in anurans, and if it occurs, we will adjust hormone dosing, timing, or freezing methods through additional trials. The new knowledge gained will provide a solid platform for adaptive management and rapid problem-solving for translation to new species and genre. Another challenge may arise if eggs from some female salamanders prove difficult to obtain, in which case we will focus on improving oocyte maturation and ovulation using hormone therapy and ultrasound. Finally, limited availability of wild males could constrain collection efforts, but by including multiple species and expanding our geographic scope, we can maintain sufficient sample sizes and resilience across regions.

INDIVIDUALS WHO WILL PLAN, IMPLEMENT AND MANAGE THE PROJECT: Dr. Andy Kouba will serve as project director for the Amphibian Conservation and Biobanking Network, with Dr. Carrie Vance serving as Co-PI and Curator of Biobanked Amphibian Collections. Both will oversee the PhD graduate student; who will work on the 4 research questions and will be assisted by several undergraduate intern students. The new human dimensions professor out of the Wildlife, Fisheries and Aquaculture Department, will serve as evaluator to the project and will assist with the formative and summative evaluations. Diane Barber (Curator Ectotherms, Fort Worth Zoo) and Jessi Krebs (Curator of Amphibians and Reptiles, Omaha Zoo) will continue to lead the two original nodes; Dustin Smith (Curator of Herpetology, Fish and Invertebrates, North Carolina Zoo) will continue as a partner and is now independently funded through AZA; Mark Vasallo (Curator of Amphibians, Detroit Zoo) will serve as the node director for the Great Lakes Region; Jennifer Hausmann (Clinical Veterinarian, Denver Zoo) will serve as director of the Rocky Mountains node. A new human dimensions faculty member at MSU will serve as **evaluator** to the project and will assist with the formative and summative evaluations. Combined this team of specialists have over 100 years of experience in amphibian husbandry, management and research; additional information can be found in the supporting documents. Each node director will supervise intern students placed at their institution. All intern students will have been trained at the MSU Hub prior to placement at the nodes, where they will be supervised by the regional directors. Our comprehensive training and mentoring plan for graduate students, undergraduate interns, and research associates can be found in the Supporting Documents as well. 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 track stored samples. We are linking our collection to the Global Genome Biodiversity Network (GGBN; <http://data.ggbn.org>) data portal with a goal to make genomic collections discoverable for research via 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, **\$749,325** 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 the MSU Hub, two Research Associates and 7 intern students at the four major regional nodes (Omaha Henry Doorly Zoo, Fort Worth Zoo, Detroit Zoo and Denver Zoo) 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. MSU and the regional zoo nodes will provide additional 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 has successfully established the foundation for rapid expansion to new 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'. *The new MSU human dimensions faculty member will assist with objective formative evaluation services for this National Leadership Program.* Their 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. 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:* Network findings will be shared broadly through peer-reviewed publications, book chapters, press releases, husbandry manuals, and popular press outlets such as *AZA Connect Magazine* and zoo newsletters. Results will also be presented at professional meetings, including the AZA annual ATAG conferences. *(2) Video Tutorials, Podcasts, Websites and Social Media:* Beyond print and conference dissemination, outreach will include six YouTube videos highlighting key technologies, regular updates via our biobanking network's social media (Instagram, Facebook), a podcast series, and yearly progress reports to AZA listservs. All video, podcast, and media resources will be accessible through the MSU Hub website at: <https://conservationphys.msstate.edu>, and will include links to node institution conservation pages. These platforms will showcase major achievements to partners, peers, and the public during and beyond the project period. *(3) Signage at Partner Institutions:* All partner zoos will develop educational signage highlighting project goals and the amphibian extinction crisis, collectively reaching more than five million visitors annually across the suite of node institutions in this project. *(4) Advanced Training Workshops:* Protocols and outcomes will be shared through on-site training for curators, veterinarians, and keepers during institutional visits. In Year 1 and 3, the AZA and Biobanking Network will host an Advanced Training Workshop on Amphibian Reproduction—tuition-based and limited to 20 participants—modeled on our previous successful workshops (see supporting documents), which may result in further recruitment of partners and creation of future nodes.

3. Project Results

INTENDED RESULTS AND HOW THEY ADDRESS THE NEED: Projected Outcomes from this national, multidisciplinary initiative will be transformative for participating institutions and the broader field. **Objective 1** will: (1) build sustainable, science-based projects that inform management; (2) strengthen program adoption through expansion of regional network nodes and shared leadership; (3) advance reproductive technologies for more amphibian species across diverse regions; (4) demonstrate the effectiveness of a *One Plan Approach* for genetic sustainability; (5) demonstrate the genetic fitness and suitability of releasing offspring into the wild that are produced through genome banking; and (6) develop an AI designed Genomic Rescue Advisor program to help with biobanking prioritization. This project extends these outcomes nationally through node placement in the Great Lakes and Rocky Mountain regions. **Objective 2** will: (1) produce curatorial resources including videos, manuals, and reports that ensure methods are *replicable and transferable*; (2) develop digital platforms enabling broad dissemination; and (3) promote these tools through social media, press, podcasts, and zoo signage. The expanded network increases both credibility and regional reach for these communications. **Objective 3** will strengthen national capacity by: (1) recruiting the next generation of curatorial and collections managers; (2) creating innovative training environments that enhance amphibian reproduction and genetic diversity; (3) advancing leadership training; and (4) establishing sustainable regional nodes that transform collections management through a One Plan framework. This project doubles the number of active nodes, generates workshops for educating an training even more practitioners with potential to recruit and expand even further nodes, realizing a truly continental network.

KNOWLEDGE, SKILLS, BEHAVIORS OR ATTITUDES THAT WILL BE CHANGED: As part of this IMLS project, 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; a new human dimensions faculty member at MSU will assist with this summative evaluation of the project. As internal evaluator, the faculty member will be responsible for the design, establishment and analysis of the summative evaluation system using the 'Qualtrics' software program. Our proposed changes focus on professional development across all audiences. We aim for **100%** of research associates and students to master assisted breeding and cryopreservation techniques for amphibians and to gain confidence as future trainers and leaders in collection stewardship. Approximately **75%** of keepers, curators, and veterinarians at partner institutions will acquire new skills in salamander assisted breeding, including handling and thawing frozen semen for IVF, and will renew their confidence in sustaining salamander populations. Finally, over **80%** of workshop participants will leave with increased knowledge of amphibian reproduction and breeding technologies and will form collaborative networks to apply and promote these methods within their institutions. This we will train an expanded cohort of professionals across more diverse geographic regions, multiplying the long-term impact on the field.

MODELS, TOOLS, RESEARCH FINDINGS AND SERVICES RESULTING FROM OUR PROJECT: This project will develop and expand 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. This project validates and scales this model across continental scope, demonstrating its applicability across diverse ecosystems and institutional contexts. 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. During the period of this grant, services will be provided *free of charge* to all zoos and aquariums across the country for biobanking specimens within their collections, consultation on reproductive services, visits, and provision of resources (supplies), with an estimated value of >\$100,000, based on previous grant periods.

SUSTAINABILITY OF THE PROJECT: A structured sustainability framework has been developed to ensure the project's continuation beyond IMLS support. It consists of seven core components: *(1) AZA and USFWS adoption:* both organizations, central to endangered species recovery, view this resource as vital to their missions. The MSU National Amphibian Genome Bank and node repositories will continue providing genetically diverse animals for collections sustainability and wild reintroductions providing long-term program support. *(2) Successful outcomes and new resources:* Participating institutions will highlight key conservation milestones (e.g., first captive breeding or wild releases) to raise visibility and attract donors. Previous successes have drawn foundation, corporate, and private support, while foundational institutions such as Fort Worth and North Carolina Zoos exemplify the model's proven sustainability with permanent new positions and additional grant funds leveraged. *(3) Capacity building and training:* Partner zoo staff—including keepers, curators, and veterinarians—will receive hands-on training in reproductive and gene-banking protocols under a “train-the-trainer” model to institutionalize program use. This project expands this through three new metropolitan zoo nodes serving as regional centers of excellence. *(4) Leadership development:* Principal investigators will mentor future professionals, including at least two research associates, one PhD student, and seven undergraduate interns who will be able advance into zoological and conservation careers. *(5) Long-term institutional commitment:* Addressing amphibian conservation has been a two-decade goal. MSU now permanently funds both Co-PIs' positions and supports the Genome Bank through its operating budget. All node zoos have likewise committed to sustaining research associate or postdoctoral positions beyond the grant period. *(6) Proven model:* The independent funding transitions of Fort Worth and North Carolina Zoos validate the Network's long-term viability, and both remain active advisors to new nodes. *(7) Funding diversification:* Future sustainability relies on multiple revenue streams—biobanking fees, annual giving, major gifts, endowments, online donations, state and NGO grants, and corporate sponsorships. Each node benefits from robust philanthropic networks and development infrastructure within their metropolitan regions.

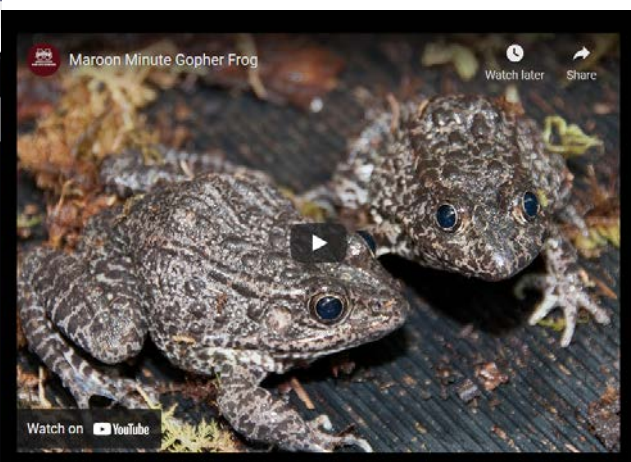
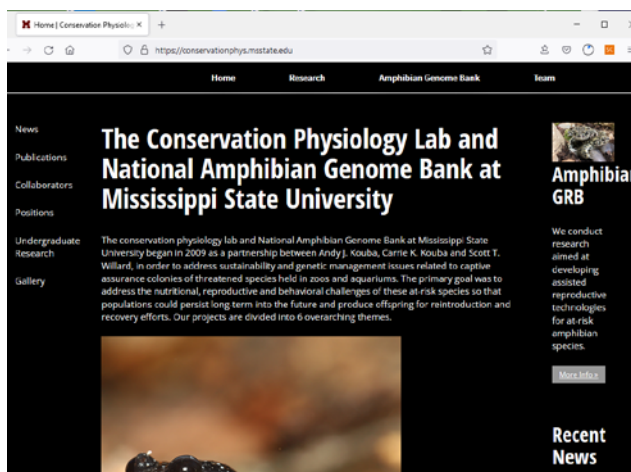
SCHEDULE OF COMPLETION :	Year 1				Year 2				Year 3			
	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
Project Set-up- Hiring personnel, Set-up of Laboratory Space, Digital output platform												
Graduate student MSU												
Research Associates at OHDZ and FWZ												
Summer Interns (7) at primary and secondary nodes												
Objective 1: <i>Foundational Research for Collections Management</i>	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
1. Research Question 1: Expanding methodologies to important collections species across genera and families. Research data collection, new protocol development gamete rescue inclusion. opportunistic sampling for emergency response												
2. Research Question 2: Linking in-situ and ex-situ populations import founder genetics												
Research team at primary and secondary nodes, wild founder lines banked, Research team at field sites, coordination with breeding seasons, new collection nodes.												
3. Research Question 3: Genetic fitness for population stewardship												
Analysis of genetic fitness of animals produced from cryopreserved sperm, PhD student												
4. Research Question 4: Integrating AI driven decision platforms for genomic data, collections sustainability. Building a Genomic Rescue Advisor that systematically ingests conservation assessments, genomic metrics, and husbandry/cryobiology information												
Objective 2: <i>Expanding Resources for Collections Stewardship</i>	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3
Establishment of a suite of resources at each U.S. regional node including in the Midwest, Southwest, Southeast, Great Lakes, Rocky Mountain and Pacific Coast regions.												
Objective 3: <i>National Capacity for Amphibian Collections Sustainability</i>	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: Training videos, imaging references, AI Advisor Platform												
Education, Training: ATAG Advanced ART Workshops, On site training of new nodes												
Information and Assessment: Outreach, Change in attitudes, One Plan Approach												
Services: consultation services provided to Zoos and aquariums for ART procedures												

Digital Products

What digital products will you create?

- **A committed Website** will be created for the Amphibian Conservation and Biobanking Network and the community partners and the public; which will be hosted by Mississippi State University. A prototype of this website can be viewed at <https://conservationphys.msstate.edu>. The website will disseminate digital products produced by the Network to create an open access resource of protocols, procedures, tutorials and reference materials for Amphibian Collections care and sustainability. The website will contain a Landing page with images, content and video links and primary pages:
 - **ABOUT US** the Amphibian Conservation and Biobanking Network and project support by IMLS
 - HUB and Node Institutions, Partners, Contributors
 - How to become a collaborator
 - **NAGB** -The National Amphibian Genome Bank **Digital Library**
 - Collection inventory available to AZA for genetic management n=1 database
 - How to check in and check out samples from the Genome Bank.
 - **RESEARCH**- Projects, Species, Outputs
 - Undergraduate, Graduate student, research Associate and Postdoc Research
 - Conferences, reports and collaborators
 - Reports (n=10)
 - Publications (n~30)
 - **RESOURCES**- Digital
 - Protocols (n=8); E-book Chapter on Collections Management (n= 1)
 - Images: Sperm image bank by species; Ultrasound image bank by species for oocyte development.
 - Tutorials, U-Tube videos, Podcasts- **How To!** Produced by the MSU TV station that will be disseminated within the zoological community on topics including:
 - Collecting sperm and eggs from frogs and toads
 - Collecting sperm and eggs from salamanders
 - How to cryopreserve and thaw amphibian sperm
 - Producing offspring through in-vitro fertilization
 - Dissection and removal of testis from deceased frogs, toads and salamanders
 - Organizing field trips for collection and cryopreservation of sperm from live wild animals.
 - **EDUCATION** and Outreach to audiences and stakeholders
 - One on one mentoring with experts.
 - Lead training workshops (similar to the one conducted in 2016)
 - **MEDIA/NEWS/HIGHLIGHTS**- rolling page of news

<https://conservationphys.msstate.edu>



- **Social Media Digital Outputs:** As a means of rapid connection with the Amphibian Community and the general public - promotion of outputs and success and links to the Website
 - Facebook (Meta)
 - Twitter
 - Instagram
 - 36 Blogs (1/month) will be created by students and research associates on success/accomplishments and new related milestones
 - Media/News/Highlights for milestones

How will you make your digital products openly available?

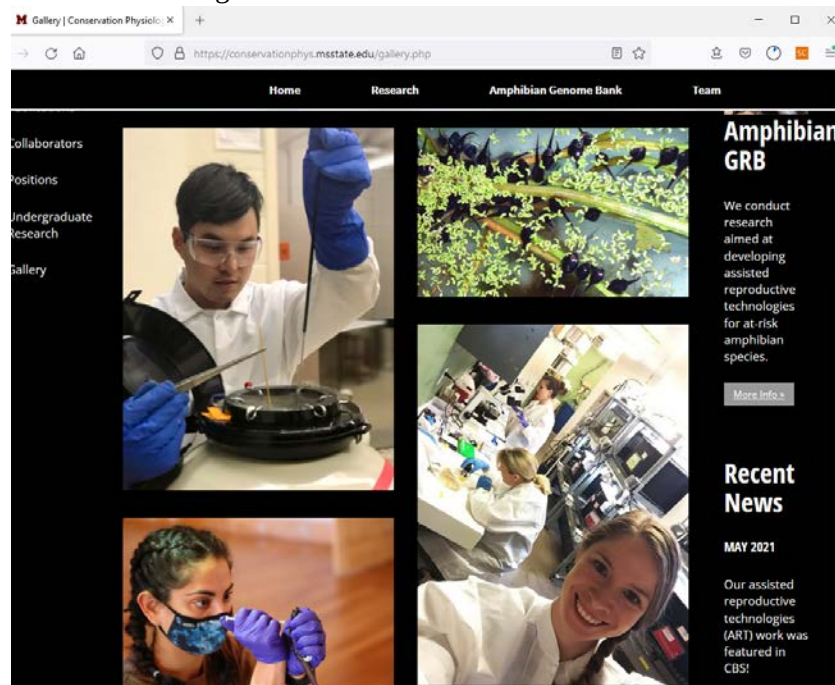
- All peer-reviewed publications will be published in open-access journals and downloadable from the Website
- All digital products (Podcasts, YouTube Videos, Images, Protocols, Database, eBook) will be openly available on-line for public access at the website.
- Accessibility will be easily available through standard web browsers (e.g. Google, Yahoo, Firefox, etc.)

What rights or limitations will you assert over your digital products?

We may share some products through a Creative Commons license and all publications must be cited if referenced. Otherwise, we do not plan to place any limitations or conditions on the intellectual property.

How will you address the sustainability of your digital products?

Materials on the website will be curated long-term and hosted on the Mississippi State University servers. These programs are tied to the long-term careers of Drs. Andy Kouba and Carrie Vance whom are at least 15 years from retirement. Should the University no longer host the programs in the future following retirements, all digital content will transfer to one of our partner AZA partner organizations that have agreed to take over management of the content and biological collections.



Public Access and Data Management Plan

1. **Peer-Reviewed publications:** We expect to publish at least 2-4 peer-review scientific articles per year in open-access journals, similar to our past publication rate and open-access practices associated with previous IMLS grant funding. IMLS will be listed in the funding source for the scope of work and project support.
2. **Provision** of a digital copy of the final proofs prior to or at publication will be provided to IMLS for each published manuscript through the designated communication portal for IMLS grant recipient services.

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=20 species, 12 males per species, 5 cryostraws per animal per year x 3 years. Total estimated banked sperm samples on average 3600 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°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°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°C Freezers are on standby in the case of power-outages, freezer malfunction and pandemics.
3. 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.
4. 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, 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.