

Sample Application

2008 National Leadership Grants for Museums

Research Category

Zoological Society of San Diego

Infectious Disease Control and Bioresource Banking for the Amphibian Extinction Crisis

Abstract

There is a global amphibian extinction crisis and zoos, aquariums and botanical gardens as well as other institutions served by the IMLS that maintain and display living amphibians have received an urgent mandate to respond on an unprecedented scale. These institutions through the “Amphibian Ark” will attempt to form captive “survival assurance colonies” to preserve up to 500 amphibian species that, without such intervention, are in imminent danger of extinction before suitable permanent solutions are found for their decline. To rapidly meet these demands unique collaborative efforts between institutions that harbor survival assurance colonies (“survival assurance partners”) will be necessary. This research proposal takes advantage of existing laboratory infrastructure and expertise at the lead institution (Zoological Society of San Diego) combined with strengths in cross-disciplinary program building at Zoo Atlanta to build a multi-institutional approach to provide urgent research and community service needs for control of infectious diseases and for establishing cell banking resources in amphibian survival assurance colonies.

The infectious disease chytridiomycosis is responsible for many of the urgent amphibian population declines worldwide that require the development of survival assurance colonies. Unfortunately, the chytrid fungus and other infectious agents such as ranaviruses are also a significant threat to the viability of the survival assurance colonies themselves and survival assurance partners nationwide require tools and resources to reduce this risk. In addition, many amphibians in survival assurance colonies will be the last representatives of their species in the world and information is urgently required to be able to establish amphibian cell culture lines that can rescue genetic information that may otherwise be lost forever.

This is a 3-year applied research and service project that will develop, provide and refine important tools necessary to create and maintain healthy and self-sustaining amphibian survival assurance colonies. Specific activities products and outcomes of this project will include:

- World Conservation Union/Conservation Breeding Specialist Group Facilitated workshops that will develop and lead to the distribution and implementation of sound, standardized, science-based and practical protocols for control of infectious diseases in amphibian survival assurance colonies. These protocols will cover quarantine, facility biosecurity, disease-risk assessment and animal transfers (between institutions and prior to release into the wild) and will be distributed widely for use by the survival assurance partners.
- Establishment of a subsidized central diagnostic testing laboratory for chytrid fungus and ranaviruses. The laboratory will encourage testing for these diseases, facilitate eradication of these population-limiting diseases from existing captive amphibian populations, and reduce the disease threat to captive survival assurance colonies.
- Collection of research data on the incidence and prevalence of key population limiting infectious diseases (e.g. chytridiomycosis and *Ranavirus* infection) that will improve protocols for control of infectious diseases and allow application of disease-risk assessment tools.
- Training of an amphibian pathology fellow to expand the availability of professionals with this expertise
- Establishment of a permanent amphibian bioresource bank in support of global research and conservation efforts as called for by the IUCN Amphibian Conservation Action Plan.

It is envisioned that this project will have substantial national impact by 1) Facilitating change in the culture and standard operating procedures of zoos, aquariums and other survival assurance partners towards practices that promote prevention of infectious diseases in amphibian collections; 2) Promoting and improving the health of amphibian survival assurance colonies nationwide by providing resources to control population-limiting infectious diseases; and 3) Meeting the infectious disease control and bioresource banking needs of survival assurance partners in a timely manner and making results readily available to the global amphibian conservation community.

Narrative

Narrative

1. Assessment of Need

“In the last decades of the 20th Century the amphibian extinction rate exceeded the mean extinction rate of the last 350 million years by at least 200 times”

IUCN Amphibian Conservation Action Plan, citing Roelants et al., 2007

An essential function of modern zoos and aquariums is the development and participation in programs that protect and reproduce endangered species of animals. These programs usually focus on a single species or groups of closely related animals (e.g. the giant panda or African and Asian elephants). In contrast, a rapidly progressing global amphibian extinction crisis is challenging these institutions to develop programs to preserve an entire vertebrate Class with over 5000 described species. The World Conservation Union (IUCN) Global Amphibian Assessment (GAA) has estimated that 32.5% of all amphibian species are threatened; up to 122 species may have become extinct since 1980; and 427 species are critically endangered (on the brink of extinction) (Stuart et al., 2004). The major contributing factor to the most drastic amphibian population declines is the disease chytridiomycosis caused by the chytrid fungus *Batrachochytrium dendrobatidis*. This fungus, disseminated worldwide by anthropogenic means, can reduce amphibian biodiversity at new locations in alarmingly short periods of time (Lips et al., 2006).

In response to the GAA findings, the Species Survival Commission (SSC) of the IUCN convened an international group of leading amphibian biologists at an Amphibian Conservation Summit resulting in the Amphibian Conservation Action Plan (ACAP) (Mendelson et al., 2006; Gascon et al., 2007). **The ACAP concluded that the amphibian extinction crisis “requires a global response at an unprecedented scale from governments, corporations, civil society and the scientific community”.** The ACAP calls for creation of “survival assurance colonies” that bring representatives of critically endangered amphibian species into captivity for safekeeping. Although remediation of factors contributing to species extinction is preferable to captivity for endangered animals, the rapidity of amphibian declines makes it likely that innumerable species will be lost long before suitable solutions are discovered. Therefore, the critical role of survival assurance colonies is to preserve the option of re-introducing species to their native habitat at a later time. The global effort to develop survival assurance colonies is coordinated by the “Amphibian Ark” a joint effort of the World Association of Zoos and Aquariums (WAZA), the IUCN/SSC, Conservation Breeding Specialist (CBSG) and Amphibian Specialist groups (www.amphibianark.org). **The Amphibian Ark estimates 500 amphibian species require immediate ex-situ intervention represented by survival assurance colonies and calls on institutions to each take responsibility for 1 or 2 of these species.** This is a massive undertaking that will affect all institutions served by the IMLS that house living amphibians including zoos, aquariums as well as some botanical gardens and Natural History museums (“survival assurance partners”). To have an effective response to this emerging challenge, collaborative efforts are essential and WAZA, Amphibian Ark and the Association of Zoos and Aquariums (AZA) have designated 2008 as “Year of the Frog” to mobilize resources for conservation, development of best practices for survival assurance colonies and public engagement. Pooling of existing individual institutional strengths and infrastructure can rapidly provide needed services and new information to all institutions participating in this effort. This proposal uses this approach to meet broad needs among the amphibian survival assurance partners for assistance in establishing best practices for control of infectious diseases and bioresource banking.

“It (chytridiomycosis) is the worst infectious disease ever recorded among vertebrates in terms of the numbers of species impacted and it’s propensity to drive them to extinction”

Amphibian Conservation Summit, Washington DC, September 2005

As efforts to establish assurance populations are initiated, it is important to recognize that the presence of chytridiomycosis and other infectious diseases in *existing amphibian collections* of survival assurance partners could threaten the viability of the assurance populations themselves. Among the most urgent issues:

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- Infections with the chytrid fungus, are lethal for many species, but in others are silent and occur in a carrier state (Daszak et al., 2004; Weldon et al., 2004; Fisher & Garner, 2007). Carrier animals act as sources of infection for species susceptible to lethal chytridiomycosis (frog versions of a “Typhoid Mary”). Often, the most susceptible species are also those that are most critically endangered (e.g. the Panamanian golden frog and Wyoming toad). The risk of subclinical infections to survival assurance colonies is illustrated by a 2007 outbreak of chytridiomycosis in Kihansi spray toads, a species likely extinct from the wild in Tanzania and that exists only in survival assurance colonies at the Bronx and Toledo Zoos. Chytridiomycosis had not previously been diagnosed in the captive populations and the source of infection was unknown. Using private funding, the entire amphibian collection housed around the toad assurance colony was tested by polymerase chain reaction (PCR) for chytrid fungus (over 300 individual animals and 20 different species) and unsuspected subclinical infections were identified in 3 species (data from Zoological Society of San Diego). Similar situations occur frequently in zoos and the risk will increase as survival assurance colonies expand. Institutions can eliminate subclinically infected animals by treatment with antifungal drugs, however, these animals can only be identified by PCR that is available from two commercial laboratories. Unfortunately, use of these labs for screening entire amphibian collections is cost-prohibitive and institutions forgo the population-wide testing needed to identify infected animals. Mass treatment of amphibian collections without PCR testing is expensive, labor-intensive and also carries risk. In addition, there has been no systematic survey of the prevalence of chytrid fungal infections in captive populations which can be used to develop less costly targeted strategies for disease surveillance (e.g. How many animals must be tested to have a 95% confidence that the collection is free of chytrid fungi or determination if certain species are more likely to be subclinical carriers)
- International amphibian trade and introduction of exotic amphibian species to non-native habitats have likely disseminated the chytrid fungus worldwide (Fisher & Garner, 2007). This is an example of “pathogen pollution” and includes movements of amphibians for incorporation into zoo collections. Zoos and other survival assurance partners are presumably leaders in wildlife conservation and ideally, should not contribute to further distribution of the chytrid fungus or other infectious agents. This distribution can occur by escape of infected collection animals; by discharge of contaminated water or cage substrates into vulnerable environments; or as the result of transfers of infected animals between different institutions (Young et al., 2007). Testing of existing captive populations for known infectious diseases like chytrid fungus would address this concern, as could standard protocols for disease control (see below). In addition, it is possible that the World Organization for Animal Health (OIE) will soon list chytrid fungus as a regulated infectious disease for international movement of amphibians (Hyatt et al., 2007). This would require survival assurance partners to widely test collection amphibians and most institutions have not or will not be able to allocate resources to comply with the new regulations.
- The potential negative impacts of infectious diseases to survival assurance colonies and management of zoo animals is not limited to the chytrid fungus. Infections by ranaviruses cause explosive die-offs of wild amphibians, but little has been done to explore the occurrence and significance of these viruses in captive collections (Daszak et al., 2003; Gascon et al., 2007; Pessier, 2008). In fact, *Ranavirus* infections may be commonly misdiagnosed because diagnostic tests (PCR) are not widely available and because postmortem findings overlap with other diseases. The possibility that *Ranavirus* infections may be transmitted between different Classes of animals such as amphibian to fish or amphibian to reptile raises concerns that undetected infections may have very broad significance for the health and management of zoo collections (Pessier, 2008). Like the chytrid fungus, *Ranavirus* infections can occur as subclinical infections with the potential for severe disease in vulnerable animals (Brunner et al., 2004). Prospective surveys of amphibian collections for ranaviruses in tissues obtained from postmortem examinations as well as outwardly healthy animals are urgently required.
- Another significant concern is the possibility that as yet unknown infectious diseases will emerge as a threat to captive (and wild) amphibian populations. Compared to other animals groups commonly housed in captive settings, amphibian medicine is an infant discipline with the first comprehensive reference published only in 2001 (Wright & Whitaker, 2001). As a result, there is an incomplete picture of the spectrum of infectious diseases in this entire Class of animals. New infectious agents with potential to impact survival assurance colonies are identified regularly, but frequently are incompletely investigated and reported (e.g. *Perkinsus*-like protozoal

disease; intravascular ciliate protozoa of Kihansi spray toads; and myxosporidiosis) (Pessier, 2008). In many cases, deaths of captive animals that have a suspected infectious etiology are undiagnosed because of a lack of resources or availability of expertise in amphibian pathology (less than 10 experts worldwide at this time). As the number of captive amphibians increase in response to the extinction crisis, more outbreaks of infectious disease will occur and this will require both laboratory capability to characterize and control these diseases as well as Veterinary Pathologists that are experts in amphibian biology and disease diagnosis.

- Until recently little attention was paid to preventative disease control measures for captive amphibian populations. As a result, best practices for quarantine (a holding period for new animals entering a collection to observe for signs of disease), disease screening (testing for specific diseases during quarantine; as part of postmortem procedures when animals die; or prior to release from collections to the wild), and biosecure work methods that reduce the possibility for disease transmission (e.g. to prevent movement of chytrid fungus or infectious agents from infected animals to uninfected animals in survival assurance colonies) have not consistently been part of the culture or standard operating procedures of amphibian keepers and collection curators. Interim guidelines for best practices have been introduced recently by the Association of Zoos and Aquariums Amphibian Taxon Advisory Group (ATAG) and by a CBSG/WAZA workshop in Panama (Zippel et al., 2006). However, these guidelines were created *ad-hoc* by a limited number of individuals in response to the urgent need and could be significantly improved by a facilitated consensus meeting of amphibian disease experts, collection curators and population managers as well as by prospective research.

“Genome resource banks can provide materials that are essential for evaluating phylogenetic systematics, genetic variability, breeding biology and dispersal patterns, health and disease, as well as serving as a tool for enhancing reproduction and rescuing genetic variation that would otherwise be lost.”

IUCN Amphibian Conservation Action Plan

To complement the development of survival assurance colonies, zoos and other survival assurance partners are well positioned to take a leading role in bioresource banking, which is another essential action step urged by the ACAP (Gascon et al., 2007). Genome resource banks (GRBs) store germplasm (such as sperm), DNA, and cell cultures from endangered animals and the contents of these banks support activities such as assisted reproduction of species, phylogenetic studies, and in extreme circumstances, reproductive cloning (Ryder et al., 2000). In many instances, the amphibians housed in the survival assurance colonies (such as the Kihansi spray toad mentioned above) will be the last representatives of their species and there is a need to preserve genetic resources from these animals. Although representatives of numerous endangered animals such as the California condor, Guam rail, great apes, among many others are present in permanent GRBs, there is a conspicuous absence of amphibian species in these repositories. In fact, only three amphibian cell culture lines have ever been reported in the scientific literature and in our experience, the procedures described need to be adapted to the range of amphibian species and the types of tissue samples likely to originate from survival assurance colonies (Ellinger et al., 1983; Kondo & Ide, 1983; Schmid et al., 2003).

Based on these needs to understand and control infectious diseases and to initiate the development of amphibian genome resource banks, this research proposal will directly benefit amphibian survival assurance partners by:

- **Providing expert consensus and science-based standardized protocols** for quarantine; infectious disease screening of captive animals; and biosecure work methods for infectious disease prevention in amphibian survival assurance colonies. Survival assurance partner curators and institutional veterinarians worldwide can confidently use these protocols to reduce the impact and risk of infectious diseases to survival assurance colonies and wild amphibian populations.
- **Providing free or at cost PCR testing for chytrid fungus and *Ranavirus* infection.** This will reduce or eliminate the financial barriers faced by survival assurance partners in instituting widespread testing for chytrid fungus and *Ranavirus* infection.
- **Providing a resource to thoroughly investigate infectious disease outbreaks in survival assurance colonies.** This easily accessible and subsidized service for survival assurance colonies will facilitate the rapid and

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comprehensive identification of new infectious diseases that put survival assurance colonies (or wild populations exposed to survival assurance colonies) at risk.

- **Providing a central communal database of infectious disease occurrence in survival assurance colonies.** This allows: 1) Veterinary advisors to survival assurance colony partners to rapidly identify trends, species predilection and occurrence of significant infectious diseases in captive populations; 2) Rapid response to significant disease events and direction of limited disease control resources to areas in which they will be most effective; and 3) Survival assurance colony partners to interface and share data with others interested in the control of amphibian infectious diseases such as the Partners in Reptile and Amphibian Conservation Chytrid Database (<http://www.parcplace.org/bdmap.html>).
- **Developing the techniques needed to create amphibian genome resource banks.** Allows survival assurance partners to extend the conservation and preservation value of amphibians already in captivity or brought into new survival assurance colonies.

2. National Impact and Intended Results

The global amphibian extinction crisis requires a rapid response from the amphibian conservation community, which includes numerous institutions served by the IMLS (e.g. zoos, aquariums, botanical gardens, among others). These institutions are responsible for forming amphibian survival assurance colonies that will provide a hedge against species extinctions and a source of animals for future reintroduction efforts. This National Leadership research proposal takes advantage of existing laboratory expertise and infrastructure at the Zoological Society of San Diego and combines these resources with a multi-institutional and multidisciplinary advisory group to provide urgent research and community service needs for infectious disease control and bioresource banking that are required for the successful establishment and maintenance of self-sustaining amphibian survival assurance colonies. Like the amphibian extinction crisis, this organized and prospective approach to specific needs of the survival assurance partner community is unprecedented and could serve as a model for future or other large-scale species preservation efforts (e.g. Turtle Survival Alliance, <http://www.turtlesurvival.org/>)

This project will have a substantial national impact by 1) Facilitating change in the culture and standard operating procedures of zoos, aquariums and other survival assurance partners towards practices that promote prevention of infectious diseases in amphibian collections; 2) Promoting and improving the health of amphibian survival assurance colonies nationwide by providing resources to control population-limiting infectious diseases; and 3) Meeting the infectious disease control and bioresource banking needs of survival assurance partners in a timely manner and making results readily available to the global amphibian conservation community. Intended results include:

- Two facilitated workshops involving key stakeholders that will develop and lead to the implementation of sound, standardized, science-based and practical protocols for control of infectious diseases in amphibian survival assurance colonies. These protocols will cover quarantine, facility biosecurity, disease-risk assessment and animal transfers (between institutions and prior to release into the wild).
- Establishment of a subsidized central diagnostic testing laboratory for chytrid fungus and ranaviruses. The laboratory will encourage testing for these diseases, facilitate eradication of these population-limiting diseases from existing captive amphibian populations, and reduce the disease threat to captive survival assurance colonies.
- Collection of research data on the incidence and prevalence of key population limiting infectious diseases (e.g. chytridiomycosis and *Ranavirus* infection) that will improve protocols for control of infectious diseases and allow application of available disease-risk assessment tools (Armstrong & Seal, 2001).
- Training of an amphibian pathology fellow to expand the availability of professionals with this expertise.
- Establishment of a permanent amphibian bioresource bank in support of global research and conservation efforts as called for by the IUCN Amphibian Conservation Action Plan.

The formal partnership of the Zoological Society of San Diego (ZSSD) and Zoo Atlanta combined with the official support and participation of the Association of Zoo and Aquariums Amphibian Taxon Advisory Group (ATAG), the Amphibian Ark, and the Conservation Breeding Specialist Group (CBSG) ensures maximal success and national

impact from this project. The department of Conservation and Research for Endangered Species (CRES) at ZSSD housed within the Arnold and Mabel Beckman Center for Conservation Research at the San Diego Wild Animal Park (<http://cres.sandiegozoo.org/>) has well-equipped and established research laboratories for investigation of wildlife diseases and conservation genetics. Key project staffs at ZSSD have extensive expertise in amphibian pathology and chytridiomycosis (Pessier), molecular diagnostic testing for animal diseases (Schrenzel), conservation genetics (Ryder), and techniques for establishing and freezing animal cell cultures for bioresource banking (Houck & Charter). The Frozen Zoo[®] within the Genetics division of CRES is an internationally renowned repository for DNA, frozen cell culture lines and germplasm from over 600 mammalian, avian and reptilian species. Using seed funding from the Annenberg Foundation, the Wildlife Disease Laboratories at ZSSD have previously offered chytrid fungus testing to zoos and wildlife agencies and have the necessary experience to offer testing on a larger scale. The formal partnership with Zoo Atlanta (Mendelson) brings input for laboratory direction from the perspective of institutional animal management, direct experience with issues related to development of survival assurance colonies, and the ability to interface fluidly with stakeholders throughout the amphibian conservation and survival assurance partner communities (Mendelson is a co-organizer of ACAP and a member of the Amphibian Ark executive committee). The cooperation of the ATAG and Amphibian Ark will encourage maximal communication with and participation of survival assurance partner institutions for purposes of gathering research samples and encouraging testing of entire amphibian collections for population-limiting diseases. The participation of CBSG is vital because of their extensive experience with group facilitation of conservation-oriented workshops that have diverse stakeholders and that are designed to systematically analyze problems and develop focused solutions using sound scientific principles. In addition, facilitation of CBSG workshops is designed to result in rapid dissemination of workshop recommendations in order to almost immediately influence stakeholders and decision makers (<http://www.cbsg.org/cbsg/workshops/>).

3. Project Design and Evaluation Plan

This is a 3-year applied research and service project that will develop, provide and refine important tools necessary to create and maintain healthy and self-sustaining amphibian survival assurance colonies in institutions served by the IMLS. Goals of the project are to: 1) Provide the research and diagnostic laboratory support necessary to successfully establish self-sustaining amphibian survival assurance colonies and successfully preserve amphibian cell culture lines; 2) Ensure that urgently needed protocols for amphibian infectious disease control are readily available to survival assurance partners and the global amphibian conservation community and 3) Establish a permanent bioresource bank for threatened and endangered amphibians. Initially, the focus of the project will be to meet urgent service needs of these institutions by providing interim expert consensus protocols for infectious disease control; making available low-cost diagnostic laboratory testing for chytrid fungus and ranaviruses; providing laboratory resources for identification of new amphibian infectious diseases; and acceptance of samples to develop amphibian cell culture techniques for genome resource banks. Later, the focus will be on improving consensus protocols for infectious disease control based on accumulated research data and on permanent preservation of amphibian cell culture lines.

The service aspects of the project will allow us to address the following research questions:

- What is the occurrence and prevalence of chytrid fungal and *Ranavirus* infections in captive amphibian collections?
- Can new infectious diseases be identified in captive amphibian collections that may be of risk to survival assurance colonies or wild amphibian populations?
- What procedures are necessary to grow and permanently preserve amphibian cell lines for use in conservation efforts?

Project Components: This project will be centered on a multi-institutional and multidisciplinary advisory group for an Amphibian Disease-Testing Laboratory (ADL) to be based in the Molecular Diagnostics Laboratory at the Beckman Center for Conservation Research, Zoological Society of San Diego. The advisory group will be formed in conjunction with the Association of Zoos and Aquariums Amphibian Taxon Advisory group (ATAG) and will be comprised of at least 11 individuals to include curators of major captive amphibian collections and leading veterinarians with an interest in amphibian disease. The advisory group will participate in amphibian disease workshops at the beginning of Year 1 and at

the end of Year 3. Sampling techniques for both chytrid fungus and *Ranavirus* testing will be non-invasive (swabs of skin and cloaca, respectively or from postmortem material). Samples for most institutions would be considered as a veterinary diagnostic procedure and not subject to Institutional Animal Care and Use Committee (IACUC) approval. However, to alleviate any animal welfare concerns, approval for this project will be sought from the Zoological Society of San Diego's IACUC prior to the first amphibian disease workshop. Samples for establishing amphibian cell culture lines will come from postmortem material.

Year 1 Activities:

1. The project will begin with a preliminary amphibian disease workshop (Disease Workshop 1) at the Zoological Society of San Diego attended by members of the project advisory group and facilitated by representatives of the CBSG. The purpose of the workshop will be to produce interim consensus-based protocols for quarantine; facility biosecurity; work methods for infectious disease control and prevention; and disease screening and surveillance for distribution to institutions housing captive amphibians. Participants will: 1) Review the *ad-hoc* guidelines previously released by the ATAG; the CBSG/WAZA Best Practices workshop (Zippel et al., 2006); the Australian Government Threat Abatement Plan for chytridiomycosis; and quarantine guidelines available on the Amphibian Diseases website (www.jcu.edu.au/school/phtm/PHTM/frogs/control.htm#quarantine); 2) Develop a strategy and guidelines for sample submission to the ADL for widespread testing of captive amphibian collections for chytrid fungus and *Ranavirus* as well as guidelines to identify and submit samples to the ADL from outbreaks of unusual or suspected infectious diseases in amphibian colonies 3) Develop standardized recommendations for antifungal treatment or handling of animals found to be subclinically infected with chytrid fungus or *Ranavirus*; 4) Produce guidelines for the entry of data into a captive amphibian disease database especially in regard to maintaining institutional privacy; 5) Produce a protocol for submission of samples to develop amphibian cell culture lines and 6) Make plans to distribute a CBSG-edited report of the workshop containing interim disease control protocols and laboratory submission guidelines to as many institutions as possible via the annual ATAG meeting (spring 2009); the ATAG listserv ("Amphibitalk"); and the Amphibian Ark website.
2. Hire two research technicians (a diagnostic laboratory technician and a research database technician) for the ADL by January 2009 and begin accessioning and processing samples for chytrid fungus and *Ranavirus* polymerase chain reaction (PCR). The research database technician will accession samples for both the ADL and the CRES Genetics Laboratory. For chytrid fungus we will use a validated Taqman quantitative PCR technique (Hyatt et al., 2007) that is the PCR test of choice for the chytrid fungus on the basis of sensitivity and internal controls for PCR inhibitors common in amphibian samples. The ZSSD has adapted this test to a 384-well format allowing for the high-throughput of samples (> 500/week) anticipated in this project. For *Ranavirus* diagnostics we will use a conventional PCR assay for a portion of the major capsid protein (MCP) gene used worldwide and that has also been established in our laboratory (Greer & Collins, 2007). After technicians are hired the ADL will begin to accept samples for chytrid fungus and *Ranavirus* PCR from all institutions that house living amphibians and charge these institutions the cost of laboratory supplies (\$15.00 for both tests compared to approximately \$50.00 for private laboratories). A pre-committed group of institutions with large amphibian programs will submit samples from all of their collection animals at no cost. This provides a set of guaranteed samples for analysis that have ensured quality control for use in gathering research data. Pre-committed institutions are the National Aquarium in Baltimore; the National Amphibian Conservation Center at the Detroit Zoological Institute; Zoo Atlanta; Atlanta Botanical Gardens; Fort Worth Zoo; and Omaha's Henry Doorly Zoo. Results from diagnostic tests will be entered into an amphibian infectious disease database (AIDD) and information communicated directly to JM at Zoo Atlanta.
3. In January 2009, using guidelines established at Disease Workshop I, the ADL will also begin accepting samples from outbreaks of suspected or unusual infectious diseases in captive amphibians. These samples could include entire fresh carcasses for postmortem examination or selected frozen or fixed tissues from dead animals. Tests that will be available and performed within the ADL to find and characterize new amphibian pathogens include gross postmortem examination; histopathology; and PCR for a wide variety of bacterial, protozoal and viral pathogens followed by DNA sequencing and BLAST analysis of positive products. Other diagnostic methods available to the ADL include clinical pathology (hematology, blood chemistry and fecal parasite analysis);

electron microscopy; and bacterial culture. Results of examinations will be entered into the amphibian infectious disease database.

4. Hire one research technician for the CRES Genetics laboratory by January 2009 and using protocols established in Disease Workshop I, begin receiving postmortem tissue samples from survival assurance colonies from institutions nationwide to establish amphibian cell culture lines. Fibroblast cell cultures will be initiated following tissue culture methods established in the Genetics laboratory for mammalian samples (Houck et al., 1994). This basic protocol has been successfully adapted and used to establish, propagate and cryopreserve viable cell cultures from over 600 taxa of mammals, birds and reptiles as well as a single amphibian cell line. A variety of growth media (MEM-alpha; L-15; Fibroblast Growth Medium; Endothelial Growth Medium; SCM1, OSCP SF9, InVitrus and Insectagro) as well as tissue types (skin, eye, tongue, gonads, heart, kidney, lung, bladder, skeletal muscle, small intestine and larynx) will be examined for potential to establish cells lines. Cell lines established during the project will be expanded and multiple aliquots cryopreserved in the Frozen Zoo[®]. One vial of each cell line will be thawed to monitor viability and for karyotype analysis (important to assess physiological and genetic health of the sample).
5. Beginning in October 2008 recruit a Veterinary Pathologist that has recently completed residency training for a 2-year fellowship in amphibian pathology and disease diagnostics. Anticipated start time for the Pathology Fellow is between July and September 2009 and the timing corresponds with the availability of potential applicants following completion of their previous training program. The Amphibian Pathology Fellow will initially be trained in basic amphibian histology and histopathology using an American Association of Museums accredited archive of pathology specimens within the Wildlife Disease Laboratories as well as amphibian pathology study sets assembled by the co-principle researcher (APP). The fellow will also be trained by staff of the Molecular Diagnostics Laboratory in performing and interpreting the results of PCR tests for chytrid fungus and ranaviruses. The Fellow will assume gradual responsibility for all aspects of the ADL under the supervision of APP.

Year 2 Activities:

1. All samples for chytrid fungus and *Ranavirus* PCR from institutions pre-committed to submitting samples will have been received and testing completed or near-completed. Results of testing will be available in the Amphibian Disease Database allowing preliminary data analysis by the Amphibian Pathology Fellow and APP. The project advisory group will review information on occurrence and prevalence of chytrid fungus and *Ranavirus* and a preliminary report issued to institutional stakeholders (e.g. the Amphibian Taxon Advisory Group meeting in Spring 2010 and American Association of Zoo Veterinarians meeting in Fall 2010).
2. Samples will continue to be accepted for chytrid fungus and *Ranavirus* PCR from institutions not previously committed to sample submission. This additional year of testing availability will allow institutions that have not already participated because of remaining financial or logistical barriers to gather these resources and perform collection wide testing. Acceptance of samples will end in October 2010.
3. Samples will continue to be accepted from outbreaks of suspected or unusual infectious diseases in captive amphibians as described for Year 1. Investigation and characterization of new amphibian pathogens by the Pathology Fellow and APP.
4. Preliminary data on chytrid fungus occurrence will be provided by JM to the USFS/PARC Chytrid Mapping Project from the Amphibian Disease Database.
5. Acceptance of postmortem tissue samples for establishing cell cultures will continue. Procedures will be adjusted as indicated by experience generated in Year 1. Established cell lines will be accessioned into the Frozen Zoo[®].

Year 3 Activities:

1. Testing will be completed on all samples for chytrid fungus and *Ranavirus* PCR with results available in the Amphibian Disease Database. The Amphibian Pathology Fellow, APP, and JM will perform a final analysis in preparation for Amphibian Disease Workshop II. Amphibian Disease Workshop II will be held at Zoo Atlanta in February 2011 with attendance of advisory group members from Workshop I. Attendees will 1) review summarized data and make decisions about updating protocols created in Workshop I; 2) Using prevalence data

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gathered for chytrid fungus and ranaviruses develop disease testing protocols for routine surveillance of captive amphibian collections that do not require testing of the entire collection; 3) Review new amphibian pathogens identified by the ADL and make recommendations for future research and collection surveillance and 4) Make plans to distribute final disease control protocols to as many institutions as possible via the annual ATAG meeting (spring 2011); the ATAG listserve (“Amphibitalk”); Final CBSG workshop report (available online) and the Amphibian Ark website.

2. Pathology Fellow and APP will prepare final reports of research findings including prevalence data for chytrid fungus and ranaviruses and descriptions of new pathogens for presentation at relevant professional meetings (AZA; AAZV; Association of Reptile and Amphibian Veterinarians) and in scientific journals (*Journal of Zoo and Wildlife Medicine; Journal of Veterinary Diagnostic Investigation; Zoo Biology*).
3. Acceptance of postmortem tissue samples for establishing cell cultures will continue. Procedures will continue to be adjusted as indicated by experience generated in Year 2. Established cell lines will be accessioned into the ZSSD Frozen Zoo[®]. Successful techniques for establishing cultures of amphibian cell lines will be prepared for scientific publication.

Evaluation Plan

Evaluation of this project is straightforward as there are numerous measurable outcomes and tangible products. These include 1) Creation of formal reports and disease control protocols as a result of Disease Workshops I and II with subsequent dissemination for use by the survival assurance colony community (see Dissemination and Sustainability); 2) Gathering of research data on disease occurrence and prevalence as well as amphibian cell banking that will be published in peer-reviewed scientific journals; 3) Evidence of multi-institutional participation in a comprehensive program to test amphibian collections for subclinical infections with chytrid fungus and ranaviruses (data will be available on number of institutions submitting samples and the number of samples evaluated) and 4) Successful accessioning of amphibian cell culture lines to the ZSSD Frozen Zoo[®].

4. Project Resources: Budget, Personnel, and Management

Staff Name	Qualifications and Commitment	Project Responsibility
Allan Pessier, DVM	Veterinary pathologist, amphibian disease specialist for 10 years, co-discover of chytrid fungus, 25% commitment each year for three years.	Project Director and Co-Principle Researcher: project supervision and budget oversight; oversees operation of amphibian diagnostic lab; supervises postdoctoral fellow; attends and participates in Disease Workshops; co-edits disease control protocols.
Joe Mendelson, PhD	Curator of Herpetology, Zoo Atlanta. Amphibian researcher (Ph.D.) with extensive work in the field of amphibian declines and conservation, including field work; cross-disciplinary program building; multi-national diplomacy; liaison across disparate institutions and organizations. 15% commitment each year for three years	Co-Principle Researcher: project supervision; liaison to survival assurance partners and amphibian conservation community; oversight of database development; joint supervision of database research technician; co-organizer and host of workshops; co-edits disease control protocols; prepares reports and protocols for dissemination
Oliver Ryder, PhD	Kleberg Chair of Genetics & Assoc. Dir. of CRES, more than 30 years of experience in conservation genetics; 10% commitment each year for three years.	Co-Principle Researcher: project supervision; oversees cell-culture sample acquisition and processing.
Marlys Houck	Cytogenetics researcher; 20 years of	Supervises cell culture laboratory; coordinates facilities

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	experience in conservation genetics; 20% commitment each year for three years.	and sample management; manages inventory database; trains staff; supervises research technician; oversees karyotype analysis.
Suellen Charter	Research coordinator; 25 years of experience with cell cultures from wildlife species; 20% commitment each year for three years.	Trains staff; troubleshoots cell culture establishment; oversees recording of acquisition information into databases; analyzes karyotypes.
Mark Schrenzel, DVM, PhD	Veterinary pathologist and molecular biologist with 15 years of experience; Head of Molecular Diagnostics; 10% commitment each year for three years.	Oversees all molecular diagnostic activities; supervises research technician; troubleshoots PCR testing and DNA sequencing; implements standardized testing protocols and oversees quality control; recommends improvements to protocols.
Tammy Tucker, MS	Senior Research technician in molecular diagnostics; 10 years of experience; 10% commitment each year for three years.	Orients and trains new laboratory technician; orders supplies; ensures laboratory diagnostic and safety standards are enforced; provides backup for new laboratory technician.
Danna Schock, PhD	Curator of Amphibians, National Amphibian Conservation Center, Detroit Zoological Society; Amphibian disease ecologist with experience in <i>Ranavirus</i> diagnostics; 1% commitment each year for three years.	Consultant on <i>Ranavirus</i> testing and biology.
Postdoctoral fellow (to be named)	Veterinary pathologist with interest in amphibian pathology. 100% commitment each year for three years.	Carries out postmortem diagnostic investigations on amphibians; compiles diagnostic data and issues reports to submitting institutions; assists with execution and interpretation of molecular diagnostic tests.
Research Technician (to be named)	Research technician with BS and experience in cytogenetics and cell-culture. 100% commitment each year for three years.	Establishes, expands, and freezes cell cultures; harvests cells, makes chromosomal preparations; prepares karyotypes; enters data.
Research Technician (to be named)	Research technician with BS and experience in molecular diagnostics. 100% commitment each year for three years.	Prepares diagnostic samples and extracts DNA for PCR testing; carries out PCR testing and DNA sequencing; compiles data and coordinates distribution of results to database manager.
Research Technician (to be named)	Research technician with BS degree (MS preferred) in field relevant to biology or computer science, and with extensive experience in developing & maintaining web-based interactive GIS informatic databases. 100% commitment each year for three years.	Receives, logs, and archives all samples; compiles data and manages database; distributes results to submitting institutions; coordinates and troubleshoots community access to the database. Develops a bioinformatics database of <i>B. dendrobatidis</i> (Bd) samples (both positive and negative) processed through the program that can be made available to relevant stakeholders and investigators, and shared with worldwide effort to map Bd.

Project Budget and Cost-Sharing: The total cost of the project for the entire three years, including administrative overhead, is \$1,260,038.33. The bulk of the expense is labor required for molecular diagnostic testing, creation of amphibian cell lines for genome resource banking, amphibian mortality investigations, and bioinformatics and bioresource database development. Supplies for diagnostic testing of existing amphibian collections at the eight core institutions, and for creation of cell lines, is an additional grant expense. The total cost for these activities is \$1,085,394.92. Administrative overhead is 29% of this amount, or \$174,643.41. Cost-sharing includes permanent staff time, costs of

hosting two workshops, and recovery of supply costs for molecular diagnostic testing (for incoming animals used to establish survival assurance colonies). The total amount requested from IMLS is \$776,862.17.

5. Dissemination

Frequent communication with institutions holding captive amphibians and amphibian survival assurance colonies will be a priority for this project. The recommendations and protocols produced at Disease Workshops I and II as well as the results of the first year of laboratory screening will be reported in preliminary form at professional meetings including the Amphibian Taxon Advisory Group (ATAG) annual meeting, the Association of Zoos and Aquariums (AZA) annual meeting and the American Association of Zoo Veterinarians (AAZV) annual meeting. Final reports and protocols produced at Disease Workshops I and II will be freely available as a downloadable PDF file from the Amphibian Ark and CBSG websites. No restrictions will be placed on access to these reports as we anticipate that they will be of value to institutions with amphibian programs internationally as well as to members of the general public that maintain and breed amphibians. Institutions with survival assurance colonies will be informed of the availability of these reports via the ATAG listserv, attendance at professional meetings (ATAG; AZA; AAZV), articles in professional newsletters such as the AZA *Connect* and CBSG newsletter and by postings on the Amphibian Ark, CBSG, ZSSD/CRES and AZA websites. Results of research findings on disease prevalence and occurrence, description of new amphibian diseases, and description of techniques for amphibian cell banking will be distributed by presentation at scientific meetings such as the AAZV and Association of Reptile and Amphibian Veterinarians annual meetings and results published in peer-reviewed journals such as the *Journal of Zoo and Wildlife Medicine*, *Zoo Biology*, *Journal of Veterinary Diagnostic Investigation* and the *Journal of Wildlife Diseases*.

6. Sustainability

This model of combining service needs with prospective research will be adaptable by other species conservation programs well into the future as population level rescue programs become more common. The reports and protocols for infectious disease control and bioresource banking established during the Disease Workshops I and II and developed in cooperation with the ATAG and other advisory groups for captive amphibian programs and survival assurance colonies are expected to become the industry standard operating procedures or “best practices”. They will be available indefinitely through the Amphibian Ark and CBSG websites as well as directly from the ATAG. Scientific publications (disease occurrence and prevalence; description of new diseases; cell banking techniques) arising from this project will remain in the public arena indefinitely. Amphibian cell lines created during this project will be accessioned permanently into the ZSSD Frozen Zoo[®] and cell lines will be made available to qualified researchers on request. Widespread testing of existing amphibian collections for important infectious diseases will facilitate elimination of major reservoirs of subclinical chytrid infections and allow institutions to more readily expand and succeed with planned survival assurance colonies. ZSSD will maintain the amphibian diagnostic lab indefinitely as long as there is a demand for the service and there is the ability to recover testing costs. If the lab is not sustainable, the research data collected during the course of this study will reduce the need for testing entire amphibian collections for disease surveillance (e.g. prevalence data allows calculation of the number of samples needed for maintenance diagnostic surveillance testing). The reduced need to submit large numbers of samples for disease surveillance, combined with changes in institutional awareness and culture in regard to important infectious diseases, will make the costs associated commercial diagnostic testing more attractive to institutions. Training of the amphibian pathology fellow will increase the availability to the zoo community of professionals with this expertise.

References

Please see supporting documents.

PARTNERSHIP STATEMENT

Complete one of these forms for each formal partner.

Legal name of applicant organization (5a from Face Sheet): Zoological Society of San Diego

1. Legal name of partner organization: Atlanta Fulton County Zoo, Inc.

2. Partner DUNS number: 174070631

3. Mailing address

Street1: 800 Cherokee Avenue, S.E.

Street2: _____

City: Atlanta

State: GA

Zip+4: 30315-1440

4. Partner Web address: http://www.zooatlanta.org

5. Partner project contact name: Joseph R. Mendelson

Title: Curator of Herpetology

Telephone number: 404-624-5655

E-mail: jmendelson@zooatlanta.org

6. Governing control of partner (choose one):

- | | |
|---|--|
| <input type="checkbox"/> State Government | <input checked="" type="checkbox"/> Nonprofit with 501(c)3 IRS Status (Other than Institution of Higher Education) |
| <input type="checkbox"/> County Government | <input type="checkbox"/> Nonprofit without 501(c)3 IRS Status (Other than Institution of Higher Education) |
| <input type="checkbox"/> City or Township Government | <input type="checkbox"/> Private Institution of Higher Education |
| <input type="checkbox"/> Special District Government | <input type="checkbox"/> Individual |
| <input type="checkbox"/> Regional Organization | <input type="checkbox"/> For-Profit Organization (Other than Small Business) |
| <input type="checkbox"/> U.S. Territory or Possession | <input type="checkbox"/> Small Business |
| <input type="checkbox"/> Independent School District | <input type="checkbox"/> Hispanic-serving Institution |
| <input type="checkbox"/> Public/State-Controlled Institution of Higher Education | <input type="checkbox"/> Historically Black Colleges and Universities (HBCUs) |
| <input type="checkbox"/> Indian/Native American Tribal Government (Federally Recognized) | <input type="checkbox"/> Tribally Controlled Colleges and Universities (TCCUs) |
| <input type="checkbox"/> Indian/Native American Tribal Government (Other than Federally Recognized) | <input type="checkbox"/> Alaska Native and Native Hawaiian Serving Institutions |
| <input type="checkbox"/> Indian/Native American Tribally Designated Organization | <input type="checkbox"/> Nondomestic (non-U.S.) Entity |
| <input type="checkbox"/> Public/Indian Housing Authority | <input type="checkbox"/> Other (specify) _____ |

7. What is the partner organization's mission? [500 characters]

We strive to inspire the citizens of Atlanta and Georgia and all visitors to the Zoo to value wildlife on Earth and to help safeguard existing species through conservation. We do this by:

- Providing an informative, educational and engaging experience;
- Being respectful and responsible stewards of the animals and the physical and financial assets entrusted to us; and
- Engaging in related conservation activities and research.

BUDGET FORM - PAGE FOUR

Section B: Summary Budget

	\$ IMLS	\$ Cost Share	\$ TOTAL COSTS
1. Salaries and Wages	407,268.00	228,774.45	636,042.45
2. Fringe Benefits	130,325.76	70,851.71	201,177.47
3. Consultant Fees	0.00	0.00	0.00
4. Travel	12,000.00	17,575.00	29,575.00
5. Supplies and Materials	52,625.00	144,545.00	197,170.00
6. Services	0.00	0.00	0.00
7. Student Support	0.00	0.00	0.00
8. Other Costs	0.00	21,430.00	21,430.00
TOTAL DIRECT COSTS (1–8)	602,218.76	483,176.16	1,085,394.92
9. Indirect Costs	174,643.41	0.00	174,643.41
TOTAL COSTS (Direct and Indirect)	776,862.17	483,176.16	1,260,038.33

Project Funding for the Entire Grant Period

1. Grant Funds Requested from IMLS	776,862.17
2. Cost Sharing:	
a. Cash Contribution	183,550.00
b. In-Kind Contribution	299,626.16
c. Other Federal Agencies*	0.00
d. TOTAL COST SHARING	483,176.16
3. TOTAL PROJECT FUNDING (1+2d)	1,260,038.33
% of Total Costs Requested from IMLS	62.00%

* If funding has been requested from another federal agency, indicate the agency's name:

Schedule of Completion

Activity/Tasks	Oct-Dec 08	Jan-Mar 09	Apr-Jun 09	Jul-Sept 09	Oct-Dec 09	Jan-Mar 10	Apr-Jun 10	Jul-Sept 10	Oct-Dec 10	Jan-Mar 11	Apr-Sept 11	IMLS Cost
Disease Screening Workshop I	█											
Introduce Project and Results of Disease Screening Workshop I to AZA Amphibian TAG		█										
Recruit laboratory technicians and Pathology Fellow	█											
Chytrid/ranavirus sample screening from pre-committed Institutions		█	█	█	█	█	█	█	█	█	█	\$72,835.00
Postmortem diagnostics				█	█	█	█	█	█	█	█	\$139,015.00
General chytrid/ranavirus diagnostics				█	█	█	█	█	█	█	█	\$101,566.76
Database development and management												\$138,401.00
Genome resource banking												\$138,401.00
Data Analysis from pre-committed institutions and report to ATAG												
Open sample acquisition and data collection		█	█	█	█	█	█	█	█	█	█	
Final data analysis									█	█	█	
Disease Workshop II												
Distribution of updated disease control protocols											█	
IMLS travel			█				█				█	\$12,000.00
Total Direct IMLS Costs												\$602,218.76