Amphibian Conservation Research Guide

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This guide is designed to be an ongoing and expanding document. Please send any comments, additions and corrections to robert.browne@gmail.com. The guide will be updated periodically on websites, and alerts of new editions sent through zoo magazines, list serves, and the Amphibian Specialist group (ASG) and the Amphibian Survival Alliance (ALA).

EXECUTIVE SUMMARY

The Amphibian Conservation Research Guide (ACRG) is an international initiative to build a framework for research that supports amphibian conservation breeding programs. To support amphibian conservation there is a need for increased research across a wide range of overlapping disciplines. The guide offers projects unique to the management of amphibians in conservation breeding programs, and also projects that deal with more fundamental aspects of biology.

We have considered zoos as focal institutions for research as they provide facilities, a means to focus the public's attention on amphibian conservation, and have a strong tradition of supporting conservation breeding programs. Research is emphasized that; 1) directly contributes to both *ex situ* and *in situ* components of amphibian conservation, 2) extends research programs widely throughout scientific fields, 3) involves the global community, 4) targets conservation breeding programs, 5) encourages collaborations, 6) directly benefits participating institutions and more broadly humanity, 7) supports young conservation scientists, and 8) develops benign research techniques.

There are many direct benefits to zoos and other institutions through engaging in amphibian research. Perhaps the most significant of these is that amphibians offer many of the best species and habitat projects for biodiversity conservation. An increasing number of conservation breeding populations are supporting species rehabitation. Amphibian research is low cost, and offers opportunities for education, display, and publicity and outreach. Amphibians in general collections and in conservation breeding programs are equally amenable to a range of research possibilities. Zoo research can involve amphibian collections in studies that improve their welfare and management, and their health and reproduction.

Amphibian conservation research can also extend to all segments of the scientific and conservation network. Zoos and other institutions can also use their populations of amphibians to provide otherwise difficult to obtain amphibians, or with biological samples including genetic or other molecular samples. In response, universities and other research institutions can engage elaborate large scale studies including physiology, behavior, reproduction, genetics, host-pathogen interactions, and pathology.

The ACRG provides projects with clearly demonstrated benefits to zoos including improved husbandry, veterinary service, reproduction rates, reduced morbidity and mortality, and improvement in genetic management. However, there are also wider benefits of amphibian conservation research including providing information to improve management, and direct benefits to humanity including the development of novel pharmaceuticals and support for ecosystem services.

Perhaps the greatest benefit amphibian conservation research can offer is the provision of research opportunities to young scientists and conservationists. In particular these programs provide a portal for young biologists to engage in research that directly provides for amphibian conservation and could lead to careers in conservation, biotechnology, and many other fields.

The ACRG investigates the current state of research and management in amphibian conservation breeding programs, discusses research potentials and how multi-disciplinary and multi-institutional projects can provide an increased resource base and greater innovation. The ACRG then provides the background to a series of research categories, their research potentials, model studies, and references and other resources.

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INTRODUCTION

The Amphibian Conservation Research Guide (ACRG) explores the research needs, priorities, and potentials of amphibian research within the framework of *ex situ* and *in situ* conservation. Particular emphasis is placed on research in zoos.

The Amphibian Conservation Action Plan (Gascon et. al. 2005) recommended conservation breeding programs to assure the survival of endangered amphibians. The needs and potentials for CBPs in zoos and other institutions are clear, and are reflected by an increasing number of amphibian CBPs globally. These CBPs reflect the broadening relationship between institutions and amphibian biodiversity conservation. CBPs extend the survival needs of amphibian species into institutions and concurrently extend the institutions' conservation programs into the field.

However, to optimize their success CBPs must be based on sound scientific knowledge and management. The background for management of amphibian research in institutions includes their scientific tradition and history, changing conservation perspectives, available facilities, and staff potential and training. Conservation breeding programs in zoos offer direct benefit to zoos through providing display, education, and potential for outreach and collaborations. Zoos can play a vital role in amphibian research through their commitment to conservation, established capacity for housing amphibians, unique collections, and their ability to publicise amphibian conservation. Amphibian research in CBPs can also directly benefit humanity through a wide range of research possibilities.

Amphibians are ideal for zoo based research. Most amphibians are small, economical and labour efficient to keep, their habitat variables are easy to modify, and many species can potentially produce hundreds to thousands of young (Burggren and Warburton 2007). Research with amphibian species novel to husbandry and for which there is little biological knowledge can be particularly fruitful. Many of the factors contributing to amphibian survival can benefit by research in CBPs. For instance, Chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (Bd), was identified as a major pathogen of amphibians in partnerships with CBPs, leading to further collaborations that developed methods for the management of Bd in captive amphibians.

Research on reproduction and larval rearing identifies methods to efficiently produce robust healthy offspring. Many amphibians have proven difficult to reproduce in captivity. Fortunately, amphibian reproduction technologies, including hormonal induction techniques have enabled the only reproduction in CPBs of many several critically endangered species. The use of biobanking, and particularly of sperm banking, are becoming necessary to both increase the efficiency of CBPs and to lower the cost of maintaining genetic variation. Providing the source populations genetic variation may a critical factor in the success of rehabilitation projects even in the case of genetic subpopulations.

Research is zoos should generally reflect the needs of biodiversity conservation and their animal's welfare. Amphibian research could increase emphasis on the husbandry, health and reproduction needs of CBPs, and amphibian research at less than 1% of all research has a lower priority than for other vertebrate groups. Nevertheless, research in zoos has made major contributions to scientific knowledge including vertebrate nutrition, reproduction, physiology, and behavior (Wemmer et al. 1997). A web search by Browne and Pereboom (2010) showed that many of the types of research necessary to support CBPs were insufficient. This particularly applied to nutrition, husbandry and environmental enrichment. Overall research in zoos tended to focus on behavior, reproduction, and animal management. However, there was some research of amphibian diet and nutrition, veterinary science, genetics, anatomy and physiology, and habitat enrichment.

Amphibians offer the potential for many zoos to establish and maintain realistic and meaningful CBPs. Amphibian CBPs offer an achievable and cost effective opportunity to maintain genetically representative populations and avoid the problems presented by the space and cost requirements of large mammals and birds (Araki et al., 2007). Amphibians can be effectively quarantined and have relatively fewer legislative and ethical restrictions on their importation than other groups. Research in zoos can offer a foundation for CBPs and can satisfy the education, display, publicity, and fundraising needs of the zoo.

There are considerable benefits to collaborations between zoos and academic institutions, including increasing their public profiles, potential for funding, scientific status, and other cultural and intellectual activities. Program implementation depends on cooperation between researchers, curators, and other staff, and research needs must be balanced with animal management, education, business, and recreation (Lawson et al. 2008). Hutchins and Thomson (2008) showed that research in zoos would benefit through prioritization using factors including the projects potential contribution to amphibian conservation, and the institutions capabilities including the ability to provide for amphibian welfare, health, longevity, and reproductive viability. Collaborations between zoos and academic institutions increase funding opportunities and are mutually beneficial (Benirschke, 1996)

The quality of amphibian research in zoos will depend on the expertise of researchers, administrative support, and institutional facilities. A credible scientific program, existing academic partnerships, and a wide range of resources, will enable zoos to attract superior candidates for research and education positions Where practicable qualified researchers should be employed to enable: 1) the dedication of resources to scientific productivity, 2) capacity building to facilitate scientific research across the organization, 3) the formation of collaborations, 4) greater access to external funding, 5) the production of high quality publications, and 6) presentations at scientific meetings (Benirschke 1996; Kleiman 1996; Lawson et al. 2008). Within this framework institutions can design a management structure for research that is tailored to their institutional capacity and amphibian collection (Hutchins 1988).

The highest ethical standards are required during amphibian conservation research. Studies of amphibians in zoos must be "*benign, non-invasive and non-intrusive and is subject to strict protocols, regulation and legal and ethical scrutiny*" (Reid et al 2008). Each research project should be approved by a research committee and an ethics committee. For a general review see 'Research Strategy of the European Association of Zoos and Aquaria (Reid et al 2008). Benign methods that can be used in zoo research include the use of images, growth and development, X-rays, thermal imaging, genetic samples, gene banking, skin secretions, blood samples, and indices of reproduction. For more information see; ILAR 2006; O'Rourke DP, Amphibians Used in Research and Teaching; Alworth and Harvey, IACUC Issues Associated with Amphibian Research.

The 'Amphibian Conservation Research Guide' does not claim to include all relevant research topics and many included topics could benefit by expansion. We have produces a number of supporting documents for unique topics or for areas of research with rapidly changing technologies that are referred to throughout the ACRG. The ACRG as an ongoing document and we appreciate any comments or input; please contact robert.browne@gmail.com

Research focus' can be broadly be divided into six groups; Husbandry projects for zoos, Conservation breeding, Rehabitation and reintroduction, *In situ* programs, Facilities, and Visitor studies.

Recommended reading.

Before studies of amphibians are undertaken a good general knowledge of amphibian biology as it relates to the study should be acquired. The following texts are recommended for scientific methods and for gaining a broad knowledge of your research area:

Books:

Dodd, CK, ed. 2009. Amphibian Ecology and Conservation, A Handbook of Techniques. Oxford University Press.

Duellman WE, Trueb L. 1994. Biology of Amphibians. Baltimore: The John Hopkins University Press.

Feder ME, Burggren WW, eds. 1992. Environmental physiology of the amphibians. University of Chicago Press.

Holfrichter R, ed. 2000. Amphibians: The world of frogs, toads, salamanders and newts. Firefly Books, Buffalo.

Institute of Laboratory and Animal Research (ILAR). Use of amphibians in the research, laboratory and classroom setting. ILAR Journal Volume 48(3).

McDiarmid, R. W., and R. Altig, eds. 1999. Tadpoles: the biology of anuran larvae. University of Chicago Press.

Ogielska M. 2009. Reproduction of Amphibians. Zoological Institute, University of Wroclaw, Poland. p 436.

Stebben RC, Cohen NW. 1995. A natural history of amphibians. Princeton University Press, Princeton.

'Amphibian Husbandry Resource Guide'. Pamuk JB, Gagliardo R 2008. Ch 1, General Amphibian Husbandry. Husbandry. Amphibian Husbandry Resource Guide. World Association of Zoos and Aquariums. pp 4-42.

ACRG supporting documents main topic folders:

- Amphibian Husbandry
- Amphibian reproduction technology
- Amphibian larval rearing
- Methods
- Zoo experiments

Use of amphibians in research, laboratory, or classroom settings In 2006 the Institute for Laboratory Animal Research (ILAR) produced a special volume on the .....

Topics with online PDFs include:

Introduction: the art of amphibian science, Smith and Stoskopf.
Amphibians Used in Research and Teaching, O'Rourke.
Facility Design and Associated Services for the Study of Amphibians, Browne et al.
Amphibian Biology and Husbandry, Pough.
Reproduction and larval rearing, Browne and Zippel.
Amphibians as Animal Models for Laboratory Research in Physiology, Burggren and Warburton.
Amphibians as Models for Studying Environmental Change, Hopkins.
IACUC Issues Associated with Amphibian Research, Alworth and Harvey.
Amphibian Resources on the Internet , Nolan and Smith.

1. HUSBANDRY PROJECTS FOR ZOOS

Perhaps the most valuable contribution many zoos can make to amphibian conservation research is through the direct use of their husbandry resources for projects. Herpetology curators and husbandry staff offer a dedicated skills base, zoos offer collections of amphibians often unique in size and variety, and zoos can directly benefit from amphibian research through display and education.

Herpetology staff can directly contribute to studies of the health, behaviour, and reproduction of resident amphibians. Their studies can also extend to increasing information available on amphibian physiology, environmental tolerance, calls, egg numbers, egg size, and hatching times, and larva development and growth. These studies when combined with global studies of amphibian ancestry and environment contribute the 'bigger picture' of amphibian biology.

1.1 Experimental design

When designing any experimental study, proper layout of the experiment (number of enclosures, number of animals per enclosure, spatial arrangement of enclosures and the experimental treatments or variables affecting them is absolutely crucial. Using the wrong layout can make all of your results impossible to analyse. It is essential to consult with someone familiar with statistical analysis and experimental design before you start an experiment. Your experiment has to be designed so that there is minimal affect on their responses by outside influences. Possible outside influences vary in their scope and potential and their consideration will depend on the type of responses you are measuring. You could send your design confidentially to someone that had previously published in the field.

These are some key considerations; 1) amphibians are very particularly plastic in some responses including growth and development, 2) animals interact with one another. Several animals in an enclosure do not make for several independent replicated measurements of the effect of a variable like food or growth, 3) animals respond to a wide range of factors, 4) some studies could include parentage as a factor and may need to run the replicates equally among the spawns of several parents, and 5) you need replicated data, and perform the correct statistical analyses on them, to see if your treatments have effects.

1.3 Development of eggs

Surprisingly there is very little recorded of the development of many amphibian species eggs and larvae. A time intensive but rewarding study is the recording of the stages of egg development and hatch. Eggs go through about 46 stages from fertilisation to egg yolk absorption. As these can occur every few hours a team of two, a record of times and image number, and a dissecting microscope with a camera is all that is needed. Take images of several eggs to make sure you have a representative sample. Each new species description of development stages is an independent article. A through literature search should be made to ascertain that the development stages have not already been published for a species before research is started.

<u>Research</u>:
1) The description of developmental times and stages for a range of species.

<u>Resources</u>:
A staging series widely used in the herpetological community is available in: Gosner, K.L. 1960. A Simplified Table for Staging Anuran Embryos and Larvae. Herpetologica, 16: 183-190. Reprinted in McDiarmid and Altig (2003) is recommend for general use.
For the specific development stages of the African clawed frog (*Xenopus laevis*) http://www-cbd.ups-tlse.fr/organismes/nieuwkoop/nieuwkoop.html

1.3 Thermal biology of amphibians (also see 1.4 and 2.3)

Thermal biology is of particular interest in amphibians as they do not maintain their temperature metabolically. Therefore, their temperature in concert with their food assimilation regulates their growth, and particularly in the case of females their ability to produce the large food stores necessary for oocyte production. The two easily studied thermoregulatory behaviours the amphibians use to maintain their preferred body temperatures are micro-habitat selection and sun basking. The relationship between temperature, food assimilation, metabolism and growth may be studied with simple equipment. Temperature can be measured with a noncontact method using a portable Handheld Infrared Thermometer. As Handheld Infrared Thermometers also measure reflected infrared, as well as the amphibians or substrate temperature, heat lamps and radiating sources of heat light like hot rocks should be off during measurements.

Resources**:**

A list of articles relating to thermal biology of amphibians and reptiles
http://www.nal.usda.gov/awic/pubs/Amphibians/amp_thermal.shtml

Amphibian optimal temperatures This supporting document provides some background and also the experimental methods needed for studies of amphibian temperature requirements.

1.3.1 Optimum temperature for eggs

The optimum temperature for the development of eggs is important in field conservation, especially in respect to climate variation and habitat modification, and also in conservation breeding programs. Previous studies have shown that hatching rate of amphibian eggs declines rapidly below and above a certain temperature plateau. This clear signal means that testing the optimum temperature for hatching eggs will yield results and can be accomplished with simple equipment. Studies made with three different spawns should be scientifically valid.

Research:
1) The determination of the optimum temperatures for eggs particularly those of threatened species, and those in CBPs.

Model studies: Goncharov et al. 1989.

Temperatures for amphibian eggs This supporting document has a simple experimental design to test the optimal hatching temperature for eggs.

1.3.2 Thermal micro-habitat selection

Amphibians' growth and reproduction is partly determined by temperature. Amphibians select different micro-habitats to maintain suitable temperature, humidity and shelter. Some species will even sun bask to maintain the highest optimal temperature. Interesting research can be done using pieces of shelter in an array, with a heat source in one area. Even the interactions between frogs can be measured.

Amphibian optimal temperatures This supporting document provides some background and also the experimental methods needed for studies of amphibian temperature requirements.

1.3.3 Sun basking

Many frogs sun bask to increase their temperature above the ambient air or substrate temperature. The preferred temperature from sun basking can simply be taken by placing frogs in a terrarium that provides a range of temperature and light environments and measuring their temperatures.

Amphibian optimal temperatures This supporting document provides some background and also the experimental methods needed for studies of amphibian temperature requirements.

Research:
1) Test temperature of sun basking frogs
2) Measure time patterns of sun basking and temperature
3) Examine the differences in growth and maturation between frogs with basking lights and those without.

Model studies: Freed 1980; Lillywhite 1970. See techniques book edited by Dodd (2009) for many more details.

1.3.4 Temperature, growth and metabolism

This study is ideal when a moderate number of anurans or salamanders must be raised. Amphibian metabolism is highly dependent on temperature and the balance between metabolism and food assimilation strongly affects growth rate. There are optimal growth temperatures for growth and development. These can be viewed as a balance between temperature and food intake that also influences growth. With the green and golden bell frog (*Litoria aurea*) at minimal temperatures growth ceases as well as food intake, in intermediate temperatures there is good growth and food conversion ratio, and at high temperatures food intake is high but growth slows. However, how far do these patterns extend into other species? The few current studies have been mainly completed with temperate pond species.

Amphibian optimal temperatures This supporting document provides some background and also the experimental methods needed for studies of amphibian temperature requirements.
Amphibian diet and nutrition This supporting document provides a general background to amphibian diet and nutrition.

Model studies: Browne and Edwards 2003.

1.4 Diet and nutrition

Nutrition has a profound effect on the growth, health and reproduction of amphibians. Limited work has been done on amphibian nutrition in general, and little effort has been made to address the needs of species with unusual metabolisms and food sources. Consequently, nutrition research offers considerable potential for surmounting many of the problems found in maintaining and reproducing amphibians in conservation breeding programs.

Research:
1) Comparison of supplement dosage and type on growth, health and reproduction.
2) Comparison of invertebrate feeds on growth, health and reproduction.
3) Assess the value of commercial nutritional regimes in conservation breeding programs.

Model studies: For adult anurans, Li et al. 2008.

Resources: Dierenfeld 1996.

Amphibian diet and nutrition This supporting document provides a general background to amphibian diet and nutrition.
Amphibian UV-B and Vitamin D_3 This supporting document provides a background to the needs for Vitamin D_3 in amphibians. It also demonstrates the equipment being used in current studies in zoos.
Amphibian live feed enrichment This supporting document provides information on the use of topical powders or dietary enrichment to improve the feeder value of crickets.

1.5 Raising of amphibian larvae

The raising of amphibian larvae is essential to maintain amphibians in conservation breeding programs and to provide amphibians for display and education. Techniques to raise amphibian larvae depend on their tendency toward cannibalism, food types, density considerations, and water quality and flow requirements. There is difficulty in reproducing many amphibian species in captivity. The main bottlenecks are through difficulties with obtaining fertile eggs and in larval raising. We have devoted a special section to obtaining fertile eggs.

In aquaculture the rearing of aquatic organisms can be categorised as high, medium and low density. Low density rearing may be necessary for species that are cannibalistic or where low levels of attention are required. Typical low density systems for anuran larvae are tadpoles at densities of one per liter or less dependent on tadpole size. Medium density is keeping tadpoles at densities of 2-6 per liter. This density requires more frequent water changes and intensity of management. High density systems can be used for some species to raise large numbers of tadpoles in small areas.

Developing the optimal rearing techniques for the larvae of various amphibian species is a valuable and rewarding experience. Several replicate tanks or trays should be trialled for each treatment and the study run for several batches of larvae for each species. An excellent primary resource for tadpole studies is McDiarmid and Altig (1999).

Model studies: Hailey et al. 2007; Hayashi et al. 2004; Browne et al. 2003. Martinez et al. 1996.

Resources:

Pramuk and Gagliardo 2008, McDiarmid and Altig 1999.

Amphibian larval rearing This supporting document provides an overview of the methods of larval rearing for amphibians.
Amphibian larval competition This supporting document provides a review of the behavioral forces mediating the growth and development of tadpoles.
Counting eggs and larvae Time saving and benign methods.
Weighing tadpoles Time saving and benign methods.

1.6 Vitamin D_3 and UV-B

Vitamin D_3 is essential to amphibians and can come from diet or from exposure to ultraviolet B radiation (UV-B) promoting Vitamin D_3 synthesis in the skin. Some amphibians such as fossorial caecilians are never exposed to UV-B, while others such as sun basking frogs are exposed to high levels. Consequently, species overall Vitamin D_3 requirements, their of UV-B requirements, their requirements for Vitamin D_3 from diet , are expected to vary between amphibian species. UV-B can have detrimental effects on both amphibians and keepers' health, the provision of UV-B is costly, and in some husbandry situations it is difficult to provide. There have been few studies of the effect of UV-B light on amphibians. The most profound responses to Vitamin D_3 deficiency are likely to be on skeletal density and deformity, and through reducing reproduction and immunity. Studies comparing UV-B levels during the rapid period of skeletal growth between metamorphosis and sub-adult should generally prove the most information, in the shortest period, for the lowest costs.

Research:
1) Develop methods for the non-invasive assessment of bone density.
2) Develop methods for the non-invasive assessment of Vitamin D_3 levels.
3) Test the effects of UV-B/ Vitamin D_3 on bone density.
4) Test the effects of UV-B/ Vitamin D_3 on amphibian health.

Model studies: None for amphibians. Reptiles: Acierno et al. 2006; Carman et al. 2000; Ferguson et al. 2005.

Resources:

http://www.uvguide.co.uk/uvinviv.htm - , a comprehensive guide to UV-B, including Vitamin D_3 synthesis, ambient levels and provision in husbandry.

Amphibian UV-B and Vitamin D_3 This supporting document provides a background to the needs for Vitamin D_3 in amphibians. It also demonstrates the equipment being used in current studies in zoos.
UV-B and Vitamin D_3 Review - Antwis and Browne (2009).

1.7 Tadpoles as predators

A particular benefit of amphibians to humanity is their role in controlling pests and disease through predation on invertebrates. One role is the predation by tadpoles reduces the number or fitness of mosquito larvae through predation, interfering with mosquito oviposition, or through food chains (Mokany 2007; Mokany and Shine 2003). In the field mosquito larvae density was 98% lower in wetlands with amphibian larvae than those without (Brodman et al. 2003). In laboratory studies tadpoles have reduced mosquito oviposition, the sizes of adult mosquitoes at emergence, and also reduced survival rates of the mosquito larvae (Hagman and Shine 2007).

Research:
1) Test the hypotheses that tadpoles might affect larval viability or oviposition behaviour of mosquitoes.
2) Net and release test whether the numbers of mosquito larvae from ponds with or without tadpoles. Associate this with other habitat variable including pond size, type and surrounding vegetation.

Resources:

Brodman 2009; Hagman and Shine 2007; Mokony 2007; Mokany and Shine 2003.

McDiarmid, R. W., and R. Altig, eds. 1999. Tadpoles: the biology of anuran larvae. University of Chicago Press.

Amphibian larval rearing This supporting document provides an overview of the methods of larval rearing for amphibians.
Counting eggs and larvae Time saving and benign methods.
Weighing tadpoles Time saving and benign methods.

2. CONSERVATION BREEDING

Conservation breeding programs for threatened species present a unique set of challenges. There are how to provide the best housing, management, and health. They concern the housing, physiology, behavior, reproduction, and other health and taxonomic decisions affecting amphibians.

2.1 Housing

Research combined with innovation has led to the design of housing systems enabling more reliable and economical husbandry of amphibians. Recently there has been a trend toward greater densities of amphibian adults and larvae housed in containers with minimal substrates. Simplified housing systems greatly increase the potential for scientific studies.

Before setting up large arrays of tanks to house amphibian populations, the experience of both zoos and private breeders should be consulted. Systems vary in their suitability for particular species, for the efficient use of space, cost, and time, for ease of maintenance, their reliability, and ease of quarantine.

Research:
1) Cost efficiency.
2) Housing and health and reproduction.
3) Automation of systems.
4) Maintenance of quarantine standards.

Model studies: Robertson et. al. 2008.

Resources:

The primary sources for information on housing and husbandry are:

Pramuk JB, Gagliardo R 2008. Ch 1, General Amphibian Husbandry. Husbandry. Amphibian Husbandry Resource Guide. Association of Zoos and Aquariums. pp 4-42.

Amphibian husbandry This folder contains a number of documents discussing amphibian housing, diet and nutrition, live feed enrichment, and environmental enrichment.
Amphibian housing This supporting document provides an overview of the housing of amphibians, particularly those necessary for the maintenance and reproduction of large number of amphibians.
Amphibian Ark quarantine standards: www.amphibianark.org; 2008. 31 p.

2.2 Management

The amphibian conservation crisis has driven improved management of amphibian conservation breeding programs. Efforts have mainly focussed on quarantine protocols, population sizes needed to maintain genetic variation, and the success of rehabitation programs. Recently there have been efforts to establish management models to link *in situ* with *ex situ* conservation programs, genetic resource banking, and global integration of amphibian conservation efforts. Recent studies have provided a range of decision making tools to choose priority species during program assessments.

Research:
1) Cost-benefit analysis of past and present amphibian conservation projects.
2) To develop improved information management systems.
3) Economic models to support amphibian conservation breeding programs.

Model studies: Lawson et al. 2008; Banks et al. 2008; Gagliardo et al. 2008; Furrer and Corredor 2008; van der Spuy and Krebs 2008.

2.2.1 Collaborations

Collaborations provide a much stronger global conservation base for amphibians. They improve both the quality and the amount of research; the linking of expertise to improves quality and the sharing of workloads increase output.

Collaboration can improve the presentation of your current and future research potentials during symposiums, exhibitions and education programs. Publicly funded research increasingly encourages the use of informal public settings to promote projects, and particularly to educate, and inspire future scientists and amphibian conservationists. Anderson et al. (2008) suggested that formal collaborations should clarify objectives, outcomes, responsibilities, and finances.

Collaborations between zoos and academic institutions could benefit from recognition of their differences in emphasis; zoo research tends to focus on welfare, conservation, display and education. Academic institutions emphasize description, experimentation, modeling, and teaching of general and specific animal biology and behavior. Fernandez and Timberlake (2008), mainly referring to mammals and birds, suggested the main fields of collaboration are the behavior and environmental enrichment, conservation breeding programs, and the education of students and the general public.

2.3 Physiology

There are a range of factors affecting the growth and development of amphibian eggs, tadpoles and adults. Temperature has a major effect on the growth and development of most amphibians. Species select their position in the environment to reach temperatures that favour growth and development of each life stage, and in nature balancing these against the environmental effects of disease and predation. These mechanisms include sun basking to increase temperature, sheltering beneath the ground during winter to avoid freezing, and for tadpoles seeking the warmth and food of the shallows on sunny days, and avoiding them on freezing nights.

The physiological requirements of amphibians must be known to enable the provision of adequate housing. For instance, amphibians will have temperature optimums for growth and reproduction. These will vary between each other and between species. For instance, some temperate species females require periods of cold, where little if any growth will occur, to bring them into breeding condition in spring. Other species live in almost constant climates with almost constant climates

The effects of climate change will threaten more species as amphibian populations become smaller and more fragmented. Studies of the effects of temperature on amphibian growth, development and survival will help predict and perhaps mitigate the effects of climate change.

2.3.1 Temperature

Growth: Amphibians are thermo-conformers or behavioral thermo-regulators. To obtain their preferred temperature behavioral thermoregulators can cryptically select microclimate, or expose themselves and actively sun bask. They also come from habitats varying from temperate climates with freezing winters and dry hot summers, to tropical climates with little diurnal or seasonal temperature variation.

Health: Unsuitable temperatures can even result in mortality of amphibians. However, temperature also has more subtle effects on amphibian health. For instance temperature affects the metabolism of vitamin D_3, essential for calcium metabolism and probably other functions, Reproduction: reproductive competence Low temperature represses spermatogenesis in some amphibians. And the development and maintenance of ovarian maturity is similarly influenced by temperature

Resources: Paniagua et al 1990. Browne and Zippel 2007.

Pramuk JB, Gagliardo R 2008. Ch 1, General Amphibian Husbandry. Husbandry. Amphibian Husbandry Resource Guide. Association of Zoos and Aquariums. pp 4-42.

Amphibian optimal temperatures This supporting document provides some background and also the experimental methods needed for studies of amphibian temperature requirements.

A list of articles relating to thermal biology of amphibians and reptiles http://www.nal.usda.gov/awic/pubs/Amphibians/amp_thermal.shtml

2.3.2 Humidity

The effects of humidity on amphibian health and survival can vary from simple comparisons of humidifying systems on husbandry conditions, to comparisons of aquatic versus terrestrial systems on the raising of salamanders. The needs of CBPs over a range of species not held in captivity include many amphibians that survive long dry periods through aestivation by burying deeply in the soils. Aestivation has been considered as necessary for to mature gonads in some species, and aestivation may also have other yet to be discovered effects. We know that some salamanders will reproduce when either kept continuously aquatic environments, or only in aquatic environments during reproduction. Which is better?

Research:
1) The effects of aquatic versus terrestrial systems on salamander health and development.
2) The effect of different humidifying systems on anuran health and development.
3) Comparing the growth, survival, and health of amphibians kept under different conditions of moisture and humidity.

Resources: Caudata.org http://www.caudata.org/cc/

Amphibian reproduction conditioning This supporting document provides an overview of environmental and nutritional requirements that promote maturation in amphibians.

2.3.3. Photoperiod

Photoperiod influences the reproductive potential, growth, reproduction, and daily skin colour and pattern change of amphibians. Increased photoperiod has been shown to increase the growth, development, and even limb regeneration rate in amphibians. Both short and long photoperiod depress spermatogenesis in some amphibians. However, there may be many other effects of photoperiod on different aspects of amphibian physiology. For instance skin pigment that was influenced by temperature was recently shown to affect mating success in some amphibians (ref).

Research:
1) Test the effect of photoperiod on amphibian growth and development.
2) Test the effect of photoperiod on amphibian skin pigment.

Model studies: Filadelfia et al. 2005; Paniagua et al. 1990; Camargo et al. 1999; Richards and Lehman, 1980.

Amphibian reproduction conditioning This supporting document provides an overview of environmental and nutritional requirements that promote maturation in amphibians.

2.3.4 Water acidity

The health and growth of amphibians is affected by water acidity. Water ranges from very acid (pH 0) to very alkaline (pH 13). Pure water is neutral and has a pH of 7. Acidic streams and bogs that provide habitat for amphibians can have a pH lower than 4. Alkaline waters of pH greater than 8 can affect amphibian survival (Odum and Zippel 2008). pH interacts with other aspects of water quality; for example some metal ions can greatly enhance the detrimental effects of low pH (see review in Alford 1999, in McDiarmid and Altig 1999). The optimal pH for the growth and development of the larvae of many species of amphibians is uncertain. This knowledge could be particularly valuable for CBPs for endangered species. Fortunately, pH is easily controlled in aquatic systems.

Research:
1) The effect of pH on larval growth and development.
2) The effect of pH on the hatching of eggs.
3) The synergistic effects of pH with other water quality parameters including temperature and toxins, and with other factors including UV-B.

2.4 Behaviour

Innovative exhibit design based on behavioural knowledge increases both amphibian wellbeing, and their value for display and education. Observations of reproductive behaviour can add to our biological knowledge of a species and enable more efficient reproduction in captivity. Even the courting and mating behaviour of many species is almost unknown. The distribution of individuals in group tanks or as individual pairs, behavioural challenges such as the playing of recorded vocalisations, and rain, have all influenced reproductive success.

Behavioural research begins with open-ended observation, which can provide basic aspects of species behaviour, and provides valuable precursors to target ‘designed’ studies to test particular factors. Studies of amphibians in zoos could contribute to wider studies. Observations of the reproductive behaviour of amphibians provide details of courtship and mating. This information may then be used to understand patterns of reproductive behaviour between different clades and environmental factors. These types of studies are already a standard part of the formation of economic theory. In 2009 the Nobel Prize for economics was shared between an economic theory on studies of resource partitioning in grazers in Mongolia, and a theory on why we are better off by forming small groups with a leader than by operating alone.

Zoos can study the autecology of species, especially, feeding, avoiding predation, selection of an optimum environment, reproduction, and fostering eggs or young. Some species of amphibians such as dendrobatids even have elaborate maternal care.

Zoos are in an ideal position to conduct behavioural studies of amphibians. In zoos long term detailed observations can be made which document aspects of behaviour that are difficult to observe in the wild. In addition the small size and minimal requirements of amphibians enable studies in easily provided habitats. Behavioural studies can also provide good publicity.

2.4.1 Proximate causes - What stimuli elicit the behaviour and how do they do it?

Research:
1) Light.
2) Sound.
3) Movement - visitor presence.
4) Husbandry disturbance.

2.4.2 Ontogeny – How did the behaviour develop, and what types of behaviour are conspicuous over the lifetime of the individual?

It is possible that amphibians raised in CBPs behave differently from those from the wild. If this involves reduced response to predation risk (movement, noise) or reduced foraging activity these behaviours could reduce their competence for rehabitation.

Research:
1) Compare the foraging ability of captive bred with wild amphibians.
2) Compare the response to light and sound of captive bred with wild amphibians.
3) Compare the response to predator threat of captive bred with wild amphibians.
4) Test response levels to stimulus levels over time.
5) Test the memory of amphibians to various stimuli.

2.4.3 Function – How does the behaviour help the individual or group survive and reproduce?

Behaviour affects larval survival and development through food consumption or increased stress. The separate raising of males and females is supposed to promote reproduction when they are introduced into conditions favourable for breeding. Predator avoidance and associated stimuli response could affect survival of amphibians (Ferrari and Chivers 2008). Research across a range of species is needed to elucidate the significance of this behavior for the fitness of adults and larvae for release.

Research:
1) Interference and exploitative competition in larvae.
2) Mating behaviour.
3) Territoriality and group behaviour.
4) Predator avoidance behaviour.

Model studies: Hurme et al. 2003.

2.4.4 Phylogeny – How did the behaviour evolve?

Research:
1) Does the behaviour correspond to phylogeny, size, or habitat variables?
2) How does behaviour develop in species suites?
3) Partitioning of calling and reproduction sites.

Model studies: Han et. al. 2007; Kats 2000.

- Intraspecific behaviour variation (Quiguango-Ubillús et al 2008).
- Relating stress to reproductive capacity (Moore and Jessop 2003).
- Relating stress to foraging activity (Crespi and Denver 2005).
- Enabling optimal mate choice to maximise fitness of offspring (Felton et al 2006; Heatwole and Sullivan 1995).
- Elucidating interesting and unique behaviours, thus increasing publicity and educational potential (Hurme et al. 2003).
- Enabling the maximum use of facilities and space to house animals (Banks et al 2008)
- Elucidating reproductive activity (Quiguango-Ubillús et al 2008; Banks et al 2008).
- Methodology, Martin and Bateson 2007, Lehner 1996; Wells 2007, Heatwole 1995, Duellman and Trueb 1994.
- Larval behaviour, McDiarmid and Altig 1999.

2.5 Reproduction

An early noteworthy research contribution on the reproduction biology of amphibians was the first breeding of the Japanese giant salamander *Andrias japonicus* by Amsterdam Zoo. The first specimen was housed in 1840 where it lived until 1881. Two other *A. japonicas* received in 1893 produced fertile eggs in 1903. By 1922 the larvae had reached about 90 cm, and one then lived until 1955. At least three Ph.D. theses derived from the breeding event, and the courtship, deposition of the eggs, and male egg guarding were described. Not until 1979 did *A. japonicas* again reproduce in captivity, in a cage in a natural stream in Asa Zoo, Hiroshima, Japan (van Bruggen, 2003).

The tradition of zoos pioneering research on amphibian reproduction has continued until the present. Traditionally these range from natural reproduction to the use of hormonal induction. Recently zoos have extended this work to elegant techniques for the manipulation of sperm and eggs; techniques that are fundamental for the maintenance of genetic variation in CBPs.

Model studies: Castillo-Trenn and Coloma 2008.

2.6 Comparing wild and captive amphibians

Banks et al 2008 conducted a study of the management and reproduction of Romer's tree frog *Chirixalus romeri.* They compared growth and reproduction between captive and wild frogs and examined the consequences of the continuous rather than seasonal reproduction of captive frogs. An interesting observation was that fecundity decreased in sequential generations of captive frogs. The extent of this phenomenon in amphibian species is uncertain as it its cause. Similar studies are suitable for many zoos.

Research:
1) Keeping records of the egg number and their viability of captive amphibians and comparing these with those of amphibians in nature.
2) Comparing the egg numbers and their viability across generations in captivity and comparing these with those of amphibians in nature.
3) Comparing the viability of

Model studies: Banks et al. 2008.

2.7 Sex diagnosis

The ability to accurately sex amphibians is essential for the management of conservation breeding programs and can provide valuable demographic information. Established methods include morphology, hormones, pheromones, behavior, calls, and ultrasound. Novel methods that hold promise are genetic sex diagnosis and hormone analysis (Szymanski et al. 2005). Groups that are particularly difficult to sex are caecilians, and also various salamanders and anurans. Sex reversal and intersex individuals can occur under conditions of captivity and pollution (Collenot et al. 1994).

2.7.1 Genetic sex diagnosis

The use of molecular techniques to sex amphibians is receiving increasing attention. However, the inheritance of sex in amphibians is complicated with a wide variety of mechanisms and in a few species sex reversal can occur (Collenot et. al. 1994). Nevertheless, sex determination in most amphibians is controlled genetically (Hayes 1998). Most amphibians lack morphologically distinguishable sex chromosomes; however, sex determining genes may provide a means to sex many species (Eggert 2004). The most promising means is identify sex specific DNA regions and diagnose sex by routine genetic techniques. Segregation analysis and linkage mapping were performed to localize an amphibian sex-determining locus in *Ambystoma* spp. (Smith and Voss

2009). Genetic sex diagnosis is economical (cost in 2009, Euro 22) and can be applied to any samples providing DNA including faeces, blood, skin, tissue or mouth swabs.

Research:
1) The development of genetic markers for the range of amphibian
2) Studies on genetic and epigenetic factors of gonadal sex differentiation in amphibians.

Model studies: Smith and Voss 2009.

Resources: Collenot et al. 1994, Hayes 1998, Smith and Voss 2009.

2.7.2 Hormonal sex diagnosis

It would be useful to be able to use hormonal sex diagnosis through fecal steroid analysis in amphibians that are difficult to sex by other methods. The only current study of hormonal sex diagnosis in amphibian species investigated species easily sexed through gross morphological differences. This study was not promising in that it showed hormonal sex diagnosis is species dependent, unreliable for one of the two species tested, and seasonally unreliable due to seasonal overlap between females and males in hormone levels. Each species under investigation will have to be easily sexed through morphology or behavior and if amphibians can be easily sexed in this way fecal analysis of sex is redundant. Another challenge for hormonal sex diagnosis is that amphibian feces are only produced occasionally, often a day to a week apart, or more. In aquatic species the use of hormonal sex diagnosis, due to leaching or break up of feces, may be even more difficult than with feces collected on land. Pheromones can be easily sampled from the skin of amphibians and may prove valuable for sex determination (Rajchard 2005).

Model studies: Fecal steroid sex determination, Szymanski et al. 2005.

2.8 Managing genetic variation

CBPs should maintain the genetic variation of the source population. This may require the maintenance of up to several hundred individuals of a single species (AArk, Guidelines for Management 2008). Biobanking offers the opportunity to secure the genetic variation of species while reducing the number of individuals required for conservation breeding programs (Mansour et al. 2009).

Concern over genetic variation can extend over the range of clades from species to sub-populations, or extending to major branches on the evolutionary tree. In 2008 genetic studies showed three distinct genetic population of the Australian, northern corroboree frog (*Pseudophryne pengilleyi)*. The conservation breeding program for *P. pengilleyi* must now maintain four populations instead of two. There were two genetic sub-populations of the Puerto Rican Crested toad (*Bufo lemur*) that had been separated for over 1 million years. Unfortunately, one sub-population not been seen in the wild since 1988, and their genetic variation has now feared lost. Genetic studies that reveal cryptic species can help the targeting of conservation efforts toward key amphibian biodiversity areas (Bickford et al. 2007).

2.7.1 Population management

<u>Model studies</u>: Bickford et al. 2007.

2.7.2 Biobanking

A biobank (biorepository) is a facility that stores and distributes biological materials and the data associated with those materials (Biobank Central 2009). Biobanking was originally formulated for human tissue depositories. However a global based biobanking system of amphibian, and associated environmental samples, holds potential for amphibian conservation.

Genetic resource banking of viable cells from sources including sperm, early eggs, and tissues from valuable deceased amphibians will be critical to the future management of genetic variation. The maintenance of genetic variation poses a challenge because of the large number of species, their genetic variation, and through management demands.

However, until the full potential of biobanking is realised we will still rely on our amphibian populations for the maintenance of genetic variation. A major challenge in conservation breeding is artificial selection for characteristics that provide high survival and early maturation in the captive environments (Akari et al. 2007). The high mortalities frequently found in larval rearing through to metamorphosis pose a particular threat of artificial selection. Whether the factors selecting larvae for survival to metamorphosis impact on juveniles and adults is unknown .

Specific sample types in biobanks include viable and unviable cells and tissues and environmental samples; including sperm, cells, nuclei, mitochondria, tissue samples, blood, substrate, stomach contents etc.

A simple and efficient method is needed to biobank the genetic variation of amphibian species. This needs to be based on current methods, and be economical and achievable with simple equipment and storage methods. The best current method appears to be the use of injection for the implantation of sperm into oocytes. Sperm can be readily sampled in the field, can be stored for days to weeks in ice, and can then be stored in -80ºC freezers. Although motility may be lost, motile sperm are not needed for sperm injection (see 2.8.4 Sperm storage and ICSI).

2.9 Reproduction technology

Reproduction technologies for amphibians have made remarkable gains during the last decade. These have mainly been driven by the conservation need to reproduce difficult species in captivity, and to maintain genetic variation in conservation breeding programs. Because of the rapid changes in this field we will regularly update our supporting documents.

Amphibian hormonal induction
Amphibian hormone cycle
Amphibian reproduction technology

Some research potentials in amphibian reproduction technologies are listed below.

2.9.1 Amphibian hormonal induction

Research:
1) Further develop techniques for the use of hormones for inducing spawning.
2) Further develop techniques for the use of hormones to sample sperm.
3) Demonstrate the biological competence of cryopreserved spermatozoa through the production of healthy offspring.

2.9.2 Short-term storage of sperm and oocytes

The short-term storage of sperm and oocytes can play plays a major role in amphibian conservation. In field situations short-term storage will enable maintenance of viability until facilities for permanent biobanking can be accessed. In conservation breeding programs the short-term storage of sperm, oocytes and other cells enables greater flexibility in the use of *in vitro* techniques including Intra-cytoplasmic sperm injection (ICSI).

The use of these techniques requires the ability to independently sample sperm from males and oocytes from females using hormones.

Research:
1) The suitability of eggs for storage at different temperatures is easily tested, because the known possible storage periods for oocytes are less than 12 hours, and the temperature ranges are between 0˚C and 35˚C. These temperatures can be maintained in most facilities using aquarium heaters and ice baths.
2) Similarly, sperm is easily kept at different temperatures and the maximum storage period tested by keeping samples in ice slurry in a refrigerator, then checking for motility using a phase contrast microscope.

2.9.3 Sperm storage and ICSI

One of the most critical needs of amphibian conservation breeding programs is the maintenance of genetic variation.

Research:
1) Develop 'field-friendly' sperm cryopreservation technologies.
2) Test ICSI techniques on sperm stored at -80˚C.
3) Develop 'field friendly' nuclei cryopreservation techniques.
4) Test ICSI techniques on nuclei stored at -80˚C.

2.10 Taxonomy

The taxonomic relationships of many amphibians are poorly known. Besides the basic requirement of species identification for biodiversity assessment, two major problems that waste resources can be alleviated by the assessment of species relationships. One is excessive effort directed toward colour varieties that have a tendency to develop in sub-populations at the extremes of species ranges. These sub-populations are also generally associated with reduced genetic variation, which makes their maintenance in conservation terms even more costly. The other problem is reduced, both *ex situ* and *in situ*, conservation efficacy in species suites that contain cryptic species. *In situ* this can result in the extirpation of a cryptic species without observable effect as its conspecific and similarly appearing congener survives, or in CBPs the possible mixing of cryptic species within the same CBP.

There are two methods that can identify cryptic species. Amphibians select mates based on calls, and different calls may lead to reproductive isolation, therefore calls can be used to discriminate cryptic species, and molecular methods can also be used to verify or discover differences. Studies of differences in amphibian calls are benign and can easily be conducted in the field or in CBPs.

Model studies: Lötters 2008, Molur 2008.

2.11 Quarantine

A central theme in CBPs is the quarantine of captive populations, particularly of rescue/supplementation programs where amphibians are potentially released in rehabitation programs.

However, the efficacy of quarantine protocols has not been adequately quantified. Zoos with quarantine facilities using various strategies are in a unique position to test how robust these facilities are. These tests can vary from fluorescent dyes to show poor of sanitary practices to testing for microbiological contamination through plating, and molecular tests for infectious agents.

Resources: Pessier 2008. Amphibian Ark quarantine standards: www.amphibianark.org; 2008. 31 p.

3. REHABILITATION

Rehabilitation (rehabitation) is establishing populations of amphibians within their biogeographical distribution in suitable biomes/habitats whether these were original, have established, or are created. The biogeographical distribution of amphibians will vary over time and is defined by factors including climate, water regimes, vegetation, predators and competitors, and disease.

The foundation of a rehabilitation program is a proposal for 'Sustainable Population Management' with this implemented through a 'Strategic Management Plan'. Amphibians would preferably be provided from extant populations; however, amphibians from Conservation Breeding Programs can also be used to provide for rehabilitation programs.

The main research emphasis in rehabilitation is the provision of large numbers of competent individuals, a suitable rehabilitation site, and assessment of the program. Competency of individuals is supported by their possessing the genetic variation of the source population, being pathogen free, of a suitable life stage, and being healthy and fit. The suitability of the rehabilitation site not only depends on the provision of suitable biotic conditions but also the absence of factors that extirpated populations originally. Assessment of success includes not only survival of the rehabilitation population but also the efficacy or detriment of the strategies involved to the rehabilitations success or failure.

3.1 Species suitability

Evaluating species suitability for rehabilitation involves factors including; 1) species historic and current range, distribution and micro-habitat, 2) reversibility of threats, 3) life history and geopolitical suitability. Species would not normally be introduced outside their historical range. However, anthropogenic habitat modification could produce novel habitat for species introductions. Supplementing existing populations should only be considers where numbers have fallen below those required for a minimum viable population and the associated risks have been assessed. The genetic variation of the supplementary population should be as close as possible to the source population. Supplementation carries the risk of both inbreeding and out-breeding depression.

<u>Research</u>:
1) The status and distribution of the species is assessed by a combination of interrogation of existing sources of information (e.g., GAA, local atlases etc.) and field survey.
2) Refinement of existing survey methodologies.

3.2 Fitness of amphibians for release

The survival of amphibians on release may be affected by husbandry and also by the life stage. Examples of release include rehabilitation programs and exotic invasions by amphibians. Examples of long running programs for the rehabilitation of Critically Endangered amphibians are the Puerto Rican Crested toad (*Bufo lemur*), the Midwife toad, and the Wyoming toad (*Bufo baxteri*) (Zippel 2009). There have also been many other successful amphibian rehabilitation programs. One early project was the rehabilitation of European tree frogs in Latvia from 1988-1992. By 2001 five generations had bred in the rehabilitation sites and 110 distinct colonies existed (Zvirgzds 1998).

Differences in the life stage, physical condition and health of amphibians affect their survival to maturity then successful reproduction. Basic population demographic data on the species needs to be gathered if these parameters are not already known, as these will be required for population viability analysis and for informing decisions about which stages of the life cycle should be used for rehabilitation. Similarly, habitat requirements will be determined so that habitat management, restoration and creation can be carried out in a way that will maximize the chances of the rehabilitation succeeding.

Research:
1) Identify restoration methods that improve amphibian habitat and population size (adaptive management).
2) Test life stages for survival.

Resources: See 2.5.1.

3.3 Disease risk assessment before release

Precautions must be taken to prevent the transmission of pathogens between amphibians in CBPs, and to naive natural populations of amphibians in rehabitation programs. CBPs require risk assessment of possible infection from the surrounding environment and the provision and observance of quarantine protocols.

However, risk assessment and quarantine protocols alone can never guarantee that animals from CBPs released in rehabitation programs are free of pathogens that may affect other amphibians. Diseases may enter the premises through air born bacteria and virus, live feeds, un-sanitised substrates and plants, human activities or through insect vectors.

Therefore, protocols need to minimize the risk of transmission of potentially invasive micro-organisms and screening should be undertaken on amphibians for release. Screening is available for Chytrid and ranavirus. However, improved screening and detection rates for these and other pathogens of concern would increase confidence in the suitability of amphibians from CBPs for rehabitation. A recent improvement is the development of DNA chips in 1993 (http://www.rochester.edu/news/show.php?id=855) and their development to the extremely sensitive (Schultz et al. 2008). The lab-on-a-chip first marketed in 2009, is capable of analyzing hundreds of samples, and multiple tests on one small chip detecting down to the levels of one bacterium (http://www.ens-newswire.com/ens/feb2009/2009-02-18-01.asp). Tests with a lab-on-a-chip can do in hours that with traditional methods would require dozens of tests and could take years to complete. The lab-on-a-chip method is more humane, much faster at 15 minutes, more sensitive and much cheaper than current tests. The use of animal tests for the presence of toxins is now obsolete (Big lab on tiny chip, Review Scientific America, October 2007, http://www.cs.virginia.edu/~robins/Big_Lab_on_a_Tiny_Chip.pdf). These chips and similar devices offer the potential for the mass screening of amphibians for all known pathogens within a decade.

Resources: Pessier 2008.

3.4 Threat mitigation

The threats leading to the decline or extinction of the species can be evaluated and neutralized following standard protocols (Caughley 1994). Where it is unlikely that some important threats to amphibians including disease or habitat loss can be negated in the immediate term then conservation breeding populations should be established and maintained. It is important that this is done before populations reach the crisis point of losing genetic variation (ref). In many species nature this is probably several thousand mature individuals. If mass pond breeding occurs in ephemeral habitats, a few males can dominate mating and the progeny of only a few females survive. This greatly reduces the effective population size resulting in population bottle necks and the rapid loss of genetic variation.

Once a species habitat requirement is known then possible rehabilitation sites should be evaluated for suitability as projects. Programs for habitat management could involve maintaining or developing existing areas, restoring areas that have lost essential habitat variables, and creation of new habitat.

3.5 Release protocols

Rehabitation most frequently involves the release of eggs, larvae and metamorphs. There has been homing behavior over several kilometers recorded in adult amphibians. Release protocols should be sensitive to any associated socio-economic risks. Instances are where the rehabitation results in reduced access to land use, particularly to traditional areas, or may impact economic production. Local communities should be stakeholders and preferably participants in any programs. Release protocols should always incorporate protocols for evaluating the success of the release in both the medium and long term time frames.

Research:

1) The relative survival after release of various life stages independently or within established populations.
2) Population and Habitat Viability Analysis (PHVA) may assist in determining targets for minimum viable populations, habitat requirements, and the time frames required to establish such populations.
3) These targets should then be embraced within a staged planning process, with interim milestones if necessary to monitor progress as the project develops.

Model studies:

Banks et. al. 2008. Romer's tree frog *Chirixalus romeri* (Hong Kong).
Drietz 2006. Wyoming toad *Bufo baxteri* (Laramie Basin, Wyoming).
Goncharov et. al. 1989. Banded newt *Triturus vittatus* (Caucasian Natural Reserve), Eastern spadefoot toad *Pelobates syriacus* (Armenia).
Oregon Spotted frog program. 2009. *Rana pretiosa* phttp://www.mtnviewconservation.org/oregon-spotted-frogs; Vancouver Zoo, http://www.gvzoo.com/node/136 ; Ministry of Environment. http://www.env.gov.bc.ca/wld/frogwatch/whoswho/factshts/orspot.htm
Puerto Rican crested toad SSP 2009. *Bufo (Peltophryne) lemur* (Puerto Rico).

3.6 Microbial fauna

Much of the microbial fauna naturally found in amphibian alimentary tracts and on their skin is beneficial for their health. In CBPs it is possible that the microbial fauna inhabiting captive amphibians will change over time. This could be due to lack of inoculation from the native habitat, and many aspects of husbandry, such as differences from natural diet and isolation from natural substrates.

These changes may lower the survival of amphibians in rehabitation programs. Very little is known of the specific roles of microbial fauna in promoting health of amphibians or protecting them from pathogens. More information is needed, including studies with a range of species comparing the survival of amphibians produced from conservation breeding programs with amphibians in nature, and elucidating the reasons underlying any differences.

3.7 Environmental contamination

Environmental contaminants may negatively impact amphibians, either through acting alone or in synergy with other stressors including habitat modification and micro-climate variation. Contamination could correspond with population declines. If this is the case targeted assays should be made of contaminant levels both in the population and in the environment. However, to reveal a direct cause then effect between contaminants and declines *ex situ* studies of toxicity must be applied. If a direct cause/effect relationship is established, and impacting levels of contaminant are found in declining populations, then further field studies are needed to show; 1) the source of the contamination, 2) the pathway of assimilation to the population, and 3) methods to ameliorate impacts on the population.

Effects of contaminants can be acute or chronic, and vary from subtle effects on health, reproduction and survival, to total and immediate death. A vast range of effects include those on behavior, growth and development, life stage survival, and fecundity. Toxicological information could be integrated into global information system (GIS) analysis of amphibian declines. However, contamination by chemicals is closely linked to industrialization and habitat clearance, which limits cause/effect relationships at larger scales.

The reasons for the difficulties in reproducing many amphibians in captivity are still obscure, and may be linked to higher contaminant levels.

Model studies: Hayes et al. 2002,

3.8 Post-release monitoring

Many amphibian species have life histories and cryptic coloration and behavior that make monitoring difficult. Often, assessment of species inventories and male population densities can only be made during the breeding season when even acoustic detection devices can automatically identify calling anuran species using a variety of call properties. Some terrestrial species can be monitored through pit trap lines and through detection through eye shine. Aquatic species can be sampled through a range of techniques of varying efficacy (Dodd 2009).

The difficulties in monitoring amphibian populations can be a major Impediment to assessing the success of rehabitation programs. Therefore, refinement and innovation in monitoring protocols will be needed, and is itself a promising topic for research. To demonstrate whether a reintroduction has resulted in the founding of self-sustaining populations, each reintroduced species will be monitored for multiple generations. Population and habitat viability analysis will be used to develop the timeframes over which 'success' can be realistically assessed using demographic and habitat data. All these activities offer exceptional opportunities, extending over long time periods, for field based researchers in conservation breeding and release.

Model studies: As for 3.5

3.9 Infectious diseases in the wild - chytrid

Infectious diseases result from the presence of pathogens including viruses, bacteria, fungi, protozoa, multi-cellular parasites, and prions. The prevention of infectious diseases has proven a substantial challenge to both *in situ* and *ex situ* amphibian conservation. In particular, the amphibian chytrid fungus heavily impacts many of the most threatened amphibians globally (Stuart et al. 2004). The amphibian chytrid has not yet been detected in many areas of high amphibian biodiversity, including many montane tropical areas similar to those in which amphibian populations have already been decimated by the amphibian chytrid, so this disease could become even more problematic (Doherty-Bone et al. 2008). Consequently, the amphibian chytrid presents a number of research needs from the molecular to the population level.

There are also a number of other pathogens that affect amphibians in the field and in captivity. In particular ranivirus' can infect field populations and can decimate populations in captivity. There are also a wide range of opportunistic bacterial and fungal pathogens that can infect and cause high mortality in captive populations.

3.9.1 *In situ* studies of the chytrid

3.9.1.1 Virulence of chytrid - phylogeny (also see 3.9.2.1)

There are is some phylogenetic clustering in susceptibility to the amphibian chytrid (*Litoria aurea* group, *Atelopus* spp.), however, the disease can have strong effects across a wide range of amphibian taxa, and any species may be vulnerable.

Increased information on phylogenetic patterns of susceptibility to chytridiomycosis could offer a means to, 1) assist in the prediction of populations declines from this cause, 2) direct studies toward characteristics that increase or decrease susceptibility to chytridiomycosis. This information can be integrated into the global information system.

<u>Research</u>:
1) List known species in respect to susceptibility to chytridiomycosis as high, moderate and no susceptibility.
2) Relate this list to amphibian phylogenetic relationships.
3) Integrate this data into unified GIS.
4) Zoos can contribute excess amphibians to institutions investigating susceptibility to Chytridiomycosis.
5) Zoos can provide samples for molecular analysis.
6) Zoos with molecular biology labs can elucidate phylogenetic relationships.

3.9.1.2 Persistence of Chytrid in the environment.

The threat of chytridiomycosis is related to its persistence in the environment; how persistent it is and what factors influence this persistence. However, little is known of the biogeographical and environmental factors associated with this. Relatively little is known about the persistence of the amphibian chytrid in the environment outside of amphibians and how this affects its impact on amphibians. Conversely, it is also not well known how amphibians affect the persistence of the amphibian chytrid in different environments.

<u>Research (general)</u>:
1) Establish more sensitive tests to detect the amphibian in environmental samples.
2) Test for the chytrid in the environment to identify sources for amphibian infection.
3) Test for the chytrid in regional environments supporting it but where unsusceptible amphibians are present (The presence of unsusceptible amphibian species may be a consequence of suite extinctions of susceptible species or of original suite resistance).

<u>Research (zoos)</u>:
1) Zoos can study environmental factors affecting the survival and persistence of chytrid - without amphibian hosts - in response to various micro-habitat variables.

3.9.1.3 Interactions with environmental change.

An understanding of the likely effects of environmental change on interactions between amphibians and the amphibian chytrid could aid in predicting future impacts on amphibian populations. From our current knowledge, it is likely that this host-pathogen relationship will be affected by changes in temperature and hydrological regimes. However, there may be other contributing factors including rainfall regimes, vector presence and abundance, and climate stochasticity.

<u>Research (general)</u>:
1) Continue research for correlates between climate change and variability and chytrid-associated amphibian declines.

Research (zoos):
1) The response of the survival and persistence of the amphibian chytrid in response to temperature, various hydrological, and vectors as anticipated by climate change scenarios.
2) The response of different strains of the chytrid to the above.

3.9.1.4 Population stability in presence of chytrid

Populations have shown varying susceptibility and resistance to chytridiomycosis. In some cases population shave been extirpated, in others a few individuals have survived (and in some cases appear to be increasing), and some populations show low but persistent infections without obvious harm. Ongoing studies through regular surveys can elucidate these processes. These require the definition of the study population, statistically designed repeatable surveys, and funding for chytrid testing.

3.9.1.5 Previous and current distribution of chytrid

There are numerous ongoing studies of the presence of the amphibian chytrid both regionally and within specific species and populations. As for 3.10.1.4 ongoing studies in neglected regions or even in locations adjacent to institutions in highly urbanized areas can contribute to pool of knowledge to assess the current distribution of chytrid. There are museums with collections of amphibian from a long historic period. These may shed light on the previous distribution of chytrid but the potential for false negatives is higher in formaldehyde preserved samples where molecular tests cannot be used.

3.9.2 *Ex situ* studies of chytrid

Studies have shown differences in the susceptibility to chytridiomycosis in respect to species reproductive strategies, life stages, micro-habitat and other biotic and physical effects. These could relate mechanisms of spread within and among populations. Habitat effects could include factors such as vectors, substrate, or water body characteristics. These variables are often recorded to various extents in autecological studies of amphibians. In addition environmental data is available from a vast number of sources and some is already available through current GIS systems. Many early studies of chytridiomycosis susceptibility were conducted in amphibian *ex situ* conservation facilities. However, within zoos, ethics guidelines prevent research that is not benign.

3.9.2.1 Phylogenetics and susceptibility to chytrid

Relationships between susceptibility to chytridiomycosis and amphibian phylogeny can be elucidated through both the development of molecular phylogenies and through studies of species susceptibility. However, confounding effects can occur through environmental variables and reproductive strategies that also affect susceptibility to chytrid. Consequently, *ex situ* studies of chytrid susceptibility where environmental variables can be controlled may be particularly suitable for collaborative studies between zoos and universities. It is already known that species can be susceptible under standard laboratory conditions that are not susceptible in nature due to environmental effects (e.g., Woodhams et al. 2003); studies examining susceptibility must decide whether they include or explicitly exclude such effects. Excluding these effects makes the studies of questionable applicability to natural populations, but including them can be difficult, since the natural thermal and humidity regimes encountered by the species examined must be known and must be simulated in the captive environment.

Model studies: Woodhams et al. 2003.

3.9.2.2 Genetic/immunology resistance to chytrid

The extant but limited relationships between phylogenetic position of amphibians and their susceptibility to chytridiomycosis, and indications that within species, some individuals may be less susceptible than others, offers the hope that there may be specific immunological factors that confer resistance. These may be associated with specific genes. The identification of such genes offers hope that in the longer term that resistance could be engineered into susceptible species. This type of technology may be of particular value for 'keystone' species that have a major role in the food chain and regulation of biomes. Recent studies have shown increased immunological competency through selection for major histocompatibility (MHC) complex genes in tadpoles, however, there was an associated trade off with reduced growth (Barribeau et al. 2008).

3.9.2.3 Probiotics and biological control

Studies have shown that dermal bacteria can reduce chytridiomycosis and also host susceptibility. Harris et al. (2009) showed that that adding an antifungal bacterial species, *Janthinobacterium lividum*, to the skins of the frog *Rana muscosa* prevented morbidity and mortality caused by chytridiomycosis. Studies to further identify candidate bacteria and their potential for probiotic mitigation of the effects of chytridiomycosis are needed. Both the interactions of bacteria with the amphibian chytrid, and the possible effects of viruses and other pathogens against chytrid, can be tested using microbiological techniques without the use of live amphibians.

Model studies: Harris et al. 2009.

3.10 Climate variation

Over geological time the earth's climate has been subject to dramatic changes in climate. Some climate scientists consider that anthropogenic factors are now causing the global climate to change at an accelerated rate on average toward global warming and that the changes will vary in extent between different regions. Many species may not be able to cope with these changes. During past periods of dramatic climatic fluctuation the survival of species has been mainly through taking refuge in small areas of suitable residual habitat, by being adaptable to a wide range of climate over a wide region, or by migrating to regions with suitable climate. With increasing fragmentation of habitat, the removal of corridors for migration, and the loss of many microhabitats, may compromise the ability of species to survive climate variation. If climate change causes excessive warming or drought some species may be particularly threatened. These include species that depend on small areas of elevation-dependent cool habitat on mountains, and those that spawn occasionally in marginal desertificating habitat.

Research is needed to predict how climate variation affects ecosystems and their associated amphibians. This can then link to information on habitat fragmentation and corridors to improve the assessment of threats and consequently the selection of species for conservation breeding programs.

4. *IN SITU* PROGRAMS

4.1 Surveys and biobanking

Research surveys can be combined with biobanking to provide information on the distribution, micro-habitat, population, and genetic variation of amphibians. Biobanks can include sperm, tissue, cells, nuclei, mitochondria and other organelles. Surveys with tissue banking provide important information and critical samples for both *in situ* and *ex situ* conservation. International cooperation provides strong incentives to all participants and should be fostered. Teams in the field can increase a projects' success through community outreach and networking. Training of volunteers, including motivational and skills training and employment support, should be incorporated where possible.

4.2 Key biodiversity sites

Establishing a Network of Conservation Sites for Amphibians - Key Biodiversity Areas (KBA) – would not only enable the efficient use of resources to conserve amphibian habitat but also could provide for; 1) baseline survey locations to assess rates of decline, 2) assessment of the presence, persistence and autecology of the amphibian Chytrid, 3) habitat fragmentation and shifting distributions, 4) increased accuracy and fine-tuning of KBA delimitations, 5) development of an adaptive strategy to deal with fluctuating populations, and 6) Integrate related research (disease, climate change, ecotoxicology) into identification of KBAs.

The KBA framework could involve research that contributes to amphibian conservation, including climatology, meteorology, mathematical modeling, environmental engineering, international law, education, and public relations, media and IT.

4.3 Community projects

Combining amphibian conservation with other conservation and environmental programs can provide increased capacity with reduced cost. Community projects include personnel training through environmental monitoring. Even temperature and rainfall are often unrecorded in important locations with unique microclimates. Community personnel can also monitor water quality and species diversity and abundance. Working with community programs for sustainability can offer important professional skills and achievements.

Communities may be interested in participating in research of the cultural, economic and biological factors affecting the conservation of a harvested species. Is there a visible relationship between the populations of harvested frogs and pests? What can be done to build a symbiosis between cultural and economic drivers and amphibian conservation? Can this be developed into an industry offering community development (Sutherland et al. 2009)?

Ecotourism, scientific tourism, education, networking, harvesting and aquaculture of amphibians offer specific activities for community projects. These can all be combined in the same project. The development of community projects for amphibian conservation offers research potentials in sustainable management, ecotourism, ecosystem services and society, and micro-economic development.

<u>Model studies</u>: Lin et al. 2008; Chang et al. 2008.

4.4 Ecotourism.

Ecotourism and scientific tourism may be welcomed by some community projects. It is a way to provide some expertise to the community and a tourism industry which can provide direct income. Both ecotourism and scientific tourism benefit through amphibian conservation research by zoos and other institutions. Field projects within many university biology courses require student engagement in accessible projects in challenging and exotic locations. Amphibian projects are very accessible, as amphibians are safe, easily observed and often diverse and abundant (Belize Project).

Model studies: Project Anuran http://www.projectanuran.org.uk/; In search of amphibians http://www.andrewgray.com/camerica/index.htm; Bride et al. 2008.

5. FACILITIES AND COLLABORATIONS

5.1 Basic husbandry facilities.

Most zoos have at least a few amphibians on display and some room off display to keep other amphibians. These facilities offer substantial potential to contribute to amphibian conservation through a variety of studies and particularly behavior and housing.

5.2 Advanced husbandry facilities

Advanced husbandry facilities include off display arrays of tanks, quarantine facilities, temperature controlled rooms, and bench space. These enable controlled studies with replicates for each treatment and the use of tanks, tubs, or trays for studies.

5.3 Zoo research centers and universities

Zoological institutions with a strong research component tend to have separate departments dedicated to research (Lawson et al. 2008). These institutions have the capacity to conduct major research projects either independently or in collaboration with other institutions. Their research directions will be a function of technical capacity, grant opportunities, species availability, expertise, and potential collaborations.

5.4 Molecular biology laboratories

Molecular biology laboratories enable studies for amphibian conservation including amphibian taxonomy, phylogeny, sex determination, and the identification of pathogens. In particular the use of molecular DNA techniques to direct the targeting of genetic sub-populations of consequence in conservation breeding programs is valuable. The development of molecular techniques to sex amphibians is a promising field of research (*see section 2.7.5.2 Hormonal sex diagnosis*).

5.5 Microbiology laboratories

Microbiological laboratories can culture and identify organisms including bacteria and viruses enabling studies of pathology, and epidermal and gut flora. Recent studies have shown that some epidermal bacteria inhibit the growth of Chytrid (Harris et. al. 2006, 2009) and may provide a prophylactic treatment. Microbiology laboratories can also be adapted for amphibian cell culture, which offers the capacity to test pathogen mechanisms of infection and corresponding immunity. Antimicrobial peptides can also be tested on cultures of the amphibian Chytrid and bacteria (Rollins-Smith and Conlon 2004) and viruses can be accessed through cell culture.

5.6 Parasitological laboratories.

Parasitological laboratories provide the opportunity to identify both pathogenic and beneficial parasites and protozoa (Desmore and Green 2007; Browne et al. 2002). The maintenance of natural gut flora in amphibians, during isolation from natural systems during long term conservation breeding programs, may affect fitness for release.

5.7 Cryobiology laboratories.

The cryopreservation of viable amphibian cells and nuclei is essential to maintaining the genetic variation of amphibians in concert with conservation breeding programs. The technical requirements of cryobiology research are relatively simple and can be accommodated in most zoos. A means for freezing cells can be provided through solid carbon dioxide (dry ice) or through -80ºC ultra-freezers. Other equipment required are phase contrast microscopes or equivalents for sperm motility assessment, cell counters including haemocytometers, and with video capture motility can be accessed through computers. The cryobiology of salamander sperm is in particular need of research (Browne and Figuel 2009).

5.8 Computer rooms

Computer rooms enable students and volunteers to research using image analysis, statistical methods and web searches. The use of digital (and video) images can enable size, shape, motility and colour comparisons. Images can be provided globally through the internet enabling broad collaborations. Literature searches enable meta-analysis and review research that can result in significant contributions to amphibian conservation.

5.9 Reproduction technologies.

A major technological challenge in conservation breeding programs is the ability to induce the production of oocytes and sperm. The induction of sperm has been reliably accomplished for many species; however, the induction of quality oocytes is less reliable. Hormones needed to research the induction of sperm and oocytes are readily available in zoos, and the assessment of efficacy is easily accomplished for sperm (see 6.3.4) and oocyte viability tested through fertility and subsequent development (Browne et al. 2006 a,b).

5.10 Veterinary laboratories.

Veterinary laboratories provide facilities that support studies of amphibian health and reproduction. X-rays can be used to reveal nutritional metabolic bone disorders or other skeletal dysfunction. Other facilities including dissecting equipment and dissecting microscopes can be used to assess pathology and most veterinary labs have ready access to histology. Veterinary laboratories also have balances, chemicals for tissue preparation, and fridges and storage mechanisms. Between X-rays, dissection, and histology with the availability of basic or advanced husbandry facilities studies of amphibian, growth, development, nutrition, health, and reproduction can be conducted.

6. VISITOR STUDIES

Education about amphibians and their conservation is becoming a major program in many zoos. Amphibians can provide examples from the conservation of individual species to conserving biodiversity and habitats. Research can elucidate the efficacy of these programs and direct the most beneficial interactions and displays to design future programs.

Zoos provide opportunities for interactions in the form of exhibit design, visitor perception and interpretation, educational, children's zoos, and public feedings (Kreger and Mench, 1995; Anderson et al., 2003). Examples of visitor interactions are demonstrations with touch tanks in aquariums and with feeding. These interactions are couched within exhibit design. Research through surveys and questionnaires' of both visitors and demonstrators, and observational studies provide a rich source of information and appeal to students from marketing to ethology.

6.1 Exhibit design

Exhibit design in zoos is in constant development to satisfy cultural, ethical and scientific perceptions of the needs of both animals and visitors. In some cases more naturalistic exhibits have been considered to provide improved education and animal welfare (Coe, 1996). Studies have shown that visitors perceive naturalistic exhibits as giving animals more freedom and promote more activity (Finlay et al., 1988). Behavior in naturalistic exhibits has also been considered to more genuinely reflect that in the field and therefore to increase visitor education including that of the need to protect habitat (Coe, 1985).

In contrast to naturalistic exhibits there is considerable potential to demonstrate aspects of animal behavior through technical and highly artificial exhibits. Examples include Perspex tunnels and glass plates to show the behavior of burrowing and fossorial animals including caecilians, and the use of complicated technical structures to display and demonstrate primate locomotion.
The ordering of exhibits also has the potential to affect visitor perception and education. Exhibits can be ordered by phylogeny, habitat type, biogeographical region, or ecological niche including convergence. Species may also be mixed in exhibits within these categories (Thomas and Maruska, 1996). There may be considerable potential for innovative display design to engage visitors. These include nocturnal, natural pond simulation, and large overhead radiated displays for sun basking species.

6.2 Visitor perception and interpretation

A major challenge to zoos is to affirm their role with visitors in maintaining the highest standards of animal welfare and in the conservation of species and their habitat. These two roles are intimately linked to anthropogenic visitor's perception of animal emotional and physical wellbeing (Reade and Waran, 1996). Because of most amphibians relatively cryptic and passive behavior their exhibits need to be designed to maximize display potential while reducing animal stress and often providing opportunity for reproduction. However, because of the small needed for amphibian displays opportunities present for strong conservation themes including technical aspects of conservation breeding programs and the challenges of the species conservation. These technical aspects include the use and development of reproductive and biotechnologies including mass rearing of larvae (Browne and Edwards 2003), and conservation challenges include habitat protection within the context of vegetation clearance, water management, disease and pollutants (Sutherland et al. 2009).

6.3 Human - animal interactions

The behaviors of the animals directly influence the behaviors of the visitors. Although many amphibians are passive during daylight, toxic species of anurans including dendrobatids, aquatic caecilians, and salamanders can be quite active. How these activities interact with visitors is unknown. However, Altman (1998) found that mammals engaging in untypical behavior promoted visitor comments more than typical behaviors and Margulis et. al. 2003 showed that activity increased visit presence.

6.4 Animal education in academia

The increasing emphasis in cellular and molecular biology has led to a paucity of student interactions with animals. Universities and other institutions in their biology programs have increased student/animal interactions through field trips often to exotic locations. Student research and internship programs in zoos offer an attractive alternative means of engaging students in animal interactions both in terms of reliability and economy. These opportunities can satisfy both academic requirements and the conservation needs of zoos. Research for students in zoos can extend from simply assisting through observations and the collection of data to full PhD research programs (Fernandez, 2001). This broad approach enables zoos to extend their education efforts through the use of their facilities by university while gaining volunteer research assistants. The success of these collaborations will create a positive feedback and encourage further collaborations.

7. CONCLUSIONS AND ACKNOWLEDGEMENTS

The main conclusion of this guide is that there are many ways that people and institutions can contribute research to support amphibian conservation. Research in zoos when combined with conservation breeding programs offers a unique platform for amphibian conservation. From this platform amphibian conservation can reach out to the general public, institutions, and to those already concerned about biodiversity conservation. One of the most important roles of amphibian research with CPBs and in zoos is its provision of a focus for the conservation aspirations of many researchers that otherwise may not directly contribute to amphibian conservation.

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WEB RESOURCES

Amphibian Ark. http://www.amphibianark.org/
Numerous pages concerning AArk activities supported by policy documents, videos, and special publications.

ARAZPA Australasian Regional Association of Zoological Parks and Aquaria.
http://www.arazpa.org.au/Year-of-the-Frog/default.aspx
The current amphibian extinction crisis is one of the greatest conservation challenges in the history of humanity. We are on the verge of losing much of an entire vertebrate class and the window of opportunity to stem the extinctions is closing. The main goal of this campaign is to generate public awareness and understanding of the amphibian extinction crisis …

British and Irish Association of Zoos and Aquariums (BIAZA) Research Guidelines
http://www.eaza.net/
This site offers a great list of subjects relating to research including ethics.

EDGE amphibians. http://www.edgeofexistence.org/amphibians/default.php
A comprehensive site that prioritises EDGE species, presents species profiles, and conservation needs and activities.

European Association of Zoos and Aquariums (EAZA). http://www.eaza.net/
EAZA Research Strategy (2008), *Chapter 10 Knowledge and Research* of the
World Zoo Conservation Strategy (WZCS; 1993), and the World Zoo and Aquarium Conservation Strategy (WZACS 2005). The BIAZA Research Guidelines provide valuable ethical perspectives.

IUCN http://www.iucn.org/what/species/wildlife/?2455
IUCN and the World Association of Zoos and Aquariums (WAZA 2005) have teamed up to increase public awareness of conservation issues and improve access to research carried out in zoos and aquariums.

IUCN Amphibian Specialist Group (ASG). http://www.amphibians.org/
A unified global response is required to tackle a crisis of this magnitude. This called for the formation of a global body ‘the Amphibian Specialist Group’ that could harness the intellectual and institutional capacity of a conservation and research network at country, regional and global levels.

Amphibian Survival Alliance http://www.amphibians.org/ The Amphibian Survival Alliance, is being established under the ASG in an attempt to conserve the world's vanishing frogs, toads and salamanders.

World Association of Zoos and Aquariums (WAZA).
http://www.waza.org/conservation/projects/index.php?main=index&view=seasia
The site displays arrays of projects by region that include frog projects.

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