Amphibian Conservation Action Plan

Proceedings: IUCN/SSC Amphibian Conservation Summit 2005

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Cover photo: Male Mallorcan midwife toad, *Alytes muletensis*, with eggs. As a result of a successful recovery program, this species was downlisted two categories from Critically Endangered to Vulnerable in 2005. Photo: Richard A. Griffiths, DICE, University of Kent, Canterbury, UK.

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Foreword

Extinction is inevitable—more than 99.9% of Earth's species are extinct (Raup 1991). David Raup went on to observe that "Extinction is a difficult research topic. No critical experiment can be performed, and inferences are all too often influenced by preconceptions based on general theory." Studying the causes of extinction has traditionally been the purview of paleontologists and not ecologists and evolutionary biologists working on contemporary systems. But accelerating losses in many species late in the 20th century have altered the scholarship of extinction by bringing the extinction events typical of evolutionary time within the dimensions of ecological time.

Beginning in the late 1980s, an especially prominent example of a global loss of biodiversity came to light as herpetologists reported amphibians had gone missing within protected parks and reserves. Since then research has shown that modern amphibian declines and extinctions have no precedent in any animal class over the last few millennia (Stuart *Et al.* 2004). About 32% of some 6000 amphibian species are threatened as compared to12% of bird and 23% of mammal species. Up to 122 amphibian species may be extinct since 1980, and population size is declining in at least 43% of species. In the last decades of the 20th century the amphibian extinction rate exceeded the mean extinction rate of the last 350 million years by at least 200 times (Roelants *et al.* 2007). Recent amphibian declines are an opportunity to study the causes of extinction in recent, not ancient, populations.

Amphibian losses have engendered research and conservation programs, and a general call to prevent more species declines and extinctions in this vertebrate class (Mendelson *et al.* 2006). Responding will require a novel, and cross-disciplinary initiative such as the Amphibian Conservation Action Plan or ACAP.

The global loss of amphibians illustrates how the world is changing, and in response conservation practices must also evolve. In the last decades of the 20th century researchers identified and promoted the conservation of local areas of great biological diversity – hotspots or regions with many endemic or otherwise distinctive species. Physical and political protection was provided for these places, but since the 1980's, field research and anecdotal observations indicate that amphibians have gone missing in diverse geographic areas and environments regardless of the protection afforded by these locations. In 1990 the Declining Amphibian The Populations Task Force (DAPTF) within the Species Survival Commission of IUCN was formed "to determine the nature, extent and causes of declines of amphibians throughout the world, and to promote means by which declines can be halted or reversed." When DAPTF was formed researchers were uncertain as to whether the disappearances were cyclical phenomena suddenly more widespread, but subsequent to the first Global Amphibian Assessment (Stuart *et al.* 2004) [GAA] the debate has shifted to understanding and mitigating the forces causing declines.

The first GAA documented the breadth of amphibian losses worldwide and made it clear that business as usual--the customary conservation approaches and practices—were not working. This realization led to the assembly in September 2005 of the Amphibian Conservation Summit convened by SSC-IUCN and Conservation International. Some 80 delegates from around the world spent four days in Washington, D.C., working on a comprehensive plan to respond to the ongoing losses of amphibian species. In addition to novel challenges such as emerging infectious diseases, toxins, and climate change, delegates also addressed familiar threats like land use change, unsustainable taking, and exotic species. The delegates acknowledged that we had a poor understanding of the complex relationships among all the factors.

The Amphibian Conservation Summit of 2005 produced a consensus among academic scientists, conservation practitioners, and knowledgeable individuals influential in diverse societal contexts (see the ACS Declaration, Appendix 1). A subset of the ACS delegation (Appendix 2) also wrote ACAP, which is a multidisciplinary approach that provides a way forward in addressing the causes of declines and slowing or reversing the losses. There is not a single answer to preventing the extinction of more species, and as a result the plan will evolve as new information becomes available. For the first time, however, in ACAP we have a response that is at the scale of the challenge. Now we need to put the plan into action.

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10 January 2007

Acknowledgements

Amphibian Conservation Summit (ACS) was a response to the alarming findings of the Global Amphibian Assessment (GAA) released in late 2004 (see www.globalamphibians.org). Over 500 scientists contributed to the GAA. The GAA, in turn, was a response to research by an untold number of scientists and concerned individuals worldwide. Consequently, more people than can be listed here are responsible for the knowledge base that has made this Action Plan possible. Many of these individuals were supported and contributed to the Declining Amphibian Populations Task Force (DAPTF) of the World Conservation Union's (IUCN) Species Survival Commission (SSC). Timothy Halliday, DAPTF's International Director for over a decade, deserves special mention for coordinating and advancing amphibian decline research around the world from 1994 to 2006. The ACS was hosted by the SSC under the leadership of Holly Dublin in coordination with Conservation International and co-chaired by James Collins, Claude Gascon, Thomas Lovejoy, Rohan Pethiyagoda, and Simon Stuart. One main result of the Summit was the creation of the Amphibian Specialist Group of IUCN-SSC (www.amphibians.org). Generous funding for the Summit was provided by the Gordon and Betty Moore Foundation. Moreover, generous contributions to conservation action within the Summit context were made by George Meyer and Maria Semple, Andrew Sabin, The Zoological Society of London and Conservation International's Critical Ecosystem Partnership Fund. Several individuals were key and played disproportionate roles that need to be acknowledged here. Don Church was the main operational hand behind the complex and comprehensive process that lead to the Summit. Mike Parr of the American Bird Conservancy graciously and expertly facilitated all three days of the ACS. Simon Stuart led the delicate and important discussions on the content of the Declaration to the ACS, enabling consensus to be reached on many complicated and controversial issues. Rohan Pethiyagoda masterfully presented the Action Plan during the Summit's plenary session on the Summit's final day. The plenary also benefited greatly from broad input by an advisory council that included Bruce Babbitt, Gonzalo Castro, Holly Dublin, Les Kauffman, Sally Lahm, Thomas Lovejoy and Russell Mittermeier. Valerie Higgins supported the logistics and smooth running of the meeting. Finally, the following people led the development of the first drafts of the white papers that served as "input" to the ACAP chapters: Ariadne Angulo, Michelle Boone, Peter Dazak, Ron Gagliardo, Richard Griffiths, Michael Lannoo, Karen Lips, Joseph Mendelson III, Gabriela Parra-Olea, Katharine Pelican, Budhan S. Pukazhenthi, Alan Pounds, Ana Carolina O.Q. Carnaval, Michael Wai Neng Lau, Oliver Ryder, Raymond Semlitsch, Debora-Leite Silvano, Simon Stuart, Gracia Syed, and David Wildt. To all these people, we wish to express our sincere thanks.

Executive Summary

The Problem

Human-induced threats to biodiversity and our natural world are varied and numerous. The manner in which our species uses the world's natural resources induces significant impacts on the rest of the species that inhabit our planet. As a species, we have continuously increased the proportion of overall primary productivity and its derivatives at the expense of the well being of most other living organisms. Through this increasing global footprint, we have decreased the amount of habitat in all biomes for the vast majority of other species, we have fragmented what little habitat remains, we have polluted most natural areas via a suite of increasingly toxic and widely used chemicals, we have depleted natural populations of many species via large-scale harvesting to the point of near extinction, we have altered the global climate of our planet through the massive burning of fossil fuels, we have created conditions that allow for wildlife diseases to cross-over and infect other taxonomic groups including our own species, and we have seen the emergence of global collapses of biological systems such as coral reefs. These impacts have been documented at different times and in different places around the world. Although many of these causal relations are direct, meaning that one type of human behavior has one particular effect, it is becoming apparent that our cumulative effect on the world's natural system is close to reaching a global tipping point. Recently, the Global Amphibian Assessment has shown that over 32% of the nearly 6,000 amphibian species known to science worldwide are at risk of going extinct. This is by far the largest proportion of an entire class of animals that is on the brink of extinction. Although many causes are at play in creating this extinction debt, a new emerging disease for amphibians (a fungal disease called chytridiomycosis) is an especially worrying cause of the disappearance of amphibians around the world. In fact, there is growing consensus among scientists that the spread of chytridiomycosis has driven and will continue to drive amphibian species to extinction at a rate unprecedented in any taxonomic group in human history. It is also possible that our global footprint as described above has created conditions that synergistically act as the "perfect storm" for amphibians to become susceptible to this new disease that is killing entire populations in the wild, and in some cases, leading to the extinction of species. The "murder by a thousand cuts" analogy is not too far fetched and amphibians, if for no other reason than their particular set of life history traits, are the first whole class of animals to be globally affected by our cumulative impact on this planet.

Without a doubt, the global amphibian extinction crisis and its present dynamics are the worst we have ever faced. For example, we know relatively little about the pathogen, such as its history or means of spreading. What we do know is that we presently have no means to control it in the wild nor do we have any proven strategies for managing amphibian populations being decimated by it. Two items of good news are that we can eliminate it from captive colonies and there are emerging prospects for tools to mitigate the effects of the disease in natural environments. We must therefore build on these successes by supporting captive breeding initiatives as a short-term response to prevent extinctions and simultaneously encourage the research and conservation programs that may ultimately open doors to reestablishing viable populations in secure habitats in the wild. Certainly, there are challenges. Many species have not been seen in years, most species have never been bred in captivity, current global capacity to support survival assurance colonies is far from adequate, and political mechanisms to facilitate an international rescue operation of hundreds of species has not been established. However, the actions required to overcome these obstacles are clear.

A third item of good news is that there is still time to save habitats for the majority of species. Whereas habitat loss remains the primary threat to amphibians worldwide and underlies most documented amphibian extinctions to date, strategic investments to safeguard critical habitats can minimize the specter of this traditional threat. Habitat conservation must remain a priority for amphibians because their usually small areas of occupancy make them more susceptible to extinction from habitat loss and degradation than other vertebrates. Furthermore, the long-term sustainability of rapid responses to save species through captive breeding and disease research rests on our capacity to preserve these species' native habitats in order to reestablish populations in the wild once we have developed the technology to do so.

The Response

The recent Global Amphibian Assessment has sharpened the scientific community's focus on both the nature and extent of threats to amphibians worldwide. This study received tremendous international coverage by the general media. Now is the time to act on new knowledge regarding the causes of an ongoing amphibian extinction event. Clear and internationally coordinated options for thwarting further extinctions of threatened amphibians must be developed with parties capable of implementing actions. The Amphibian Conservation Summit was called because it is morally irresponsible to document amphibian declines and extinctions without also designing and promoting a response to this global crisis. To this end, the Amphibian Conservation Summit has designed the Amphibian Conservation Action Plan (ACAP) that is here presented in its initial form, and commends it to governments, the business sector, civil society and the scientific community for urgent and immediate adoption and implementation.

A steering committee convened in September 2005 to set priorities for conservation and research actions within nine thematic areas relevant to amphibian conservation; 1) Designing a network of conservation sites for amphibians—Key Biodiversity Areas; 2) Freshwater resources and associated terrestrial landscapes; 3) Climate change, biodiversity loss, and amphibian declines; 4) Infectious diseases; 5) Over-harvesting of amphibians; 6) Evaluating the role of environmental contamination in amphibian population declines; 7) Captive programs; 8) Reintroductions;

Theme	Five-year Budget (US$)
Key Biodiversity Areas	120,000,000
Freshwater Resources and Terrestrial Landscapes	125,000,000
Climate Change	7,360,000
Infectious Diseases	25,455,000
Over-harvest	4,300,000
Environmental Contamination	43,190,000
Captive Programs	41,994,000
Reintroductions	4,000,000
Assessment	1,850,000
Systematics	32,150,000
Bioresource Banking	4,000,000
Total	409,299,000

9) The continuing need for assessments: making the Global Amphibian Assessment an ongoing process; 10) Systematics and conservation; and 11) Bioresource banking efforts in support of amphibian conservation.

A declaration was released following the Summit urging four kinds of intervention that are needed to conserve amphibians, all of which need to be started immediately:

1. Expanding our understanding of the causes of declines and extinctions
2. Continuing to document amphibian diversity, and how it is changing
3. Developing and implementing long-term conservation programmes
4. Responding to emergencies and immediate crises

The full Declaration is in Appendix 1

The ACAP is the most ambitious program ever developed to combat the extinction of species, reflecting the reality that the amphibian extinction crisis requires a global response at an unprecedented scale. The ACAP requires the international community to enter uncharted territory and to take great risks. But the risks of inaction are even greater. The ACAP calls on all governments, corporations, civil society and the scientific community to respond. There needs to be unprecedented commitment to developing and implementing the ACAP with accompanying changes in international and local environmental policies that affect this class of vertebrate animals—as they truly are the proverbial canaries in the global coal mine. This document offers practical, large-scale, creative, innovative and realistic actions that will be required to halt the present tide of extinctions of amphibian species and includes an ambitious yet realistic budget.

A unified global strategy incorporating survival assurance colonies, disease research, and habitat protection forms the focus of this new plan to save amphibians. We must, of course, also remain vigilant and act on other threats, including climate change, over-harvesting, and toxins. Although they may not act as swiftly as the aforementioned factors, extinction can occur as a consequence of these other threats. For instance, pollution led to the extinction of Kunming Lake newt (*Cynops wolterstorffi*), climate change contributed to the extinction of the Costa Rican golden toad (*Bufo periglenes*), and over-harvesting is severely threatening several of China's remarkable frogs and salamanders. Furthermore, research on the interactions between amphibians and these threats allows us to detect the early warning alarms to environmental problems that amphibian populations can sound so well. Lessons learned from confronting the amphibian crisis may be transferable to other groups and ecosystems. We have many other potential crises-in-the-making such as coral reef collapses, fisheries collapses, emerging human diseases such as Ebola, SARS, Nipah virus, and our general management of freshwater that will certainly lead to global shortages of potable drinking water with its obvious consequences. Many if not all of these environmental time bombs are the result to varying degrees of the same human footprint that our species is having on this planet.

The road to success must include a broad set of stakeholders that can aid in the implementation of the ACAP. This is important not only because there are many issues that are beyond the simple realm of "amphibian conservation work", but also because it is very possible that addressing many of the underlying causes of this crisis will help us avert the next global environmental catastrophe. Helping curb unsustainable wildlife use would not only decrease some of the threats on particular amphibian species, but help us apply these same solutions to other species as well (tigers, birds, etc.). Similarly, tackling climate change, although a huge task in its own right, will make a huge contribution to the continued survival of all species as well as to the sustainability of life support systems in general.

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Summary of Action Steps

The ACAP is designed to provide guidance for implementing amphibian conservation and research initiatives at all scales from global down to local. Whether it is local NGOs seeking to incorporate amphibians into management plans of protected areas or devise a regional or national strategy for amphibian conservation, governments seeking to fulfill their CBD 2010 targets, or researchers working to fill crucial gaps in knowledge, the ACAP aims to provide clear guidance on the most pertinent issues. It's breadth in scope should not discourage individuals wishing to implement amphibian conservation actions on a small scale or covered by a small subset of the ACAP. To assist with the practical implementation of the ACAP, this chapter distills the action points from each chapter into a user-friendly guide. Stakeholders from conservation practitioners to researchers to government officials may glean from this the actions that are applicable and refer to relevant chapters for more depth on any of the themes.

To provide an example of its implementation, the ACAP has been adopted as a framework for developing National Amphibian Action Plans in Madagascar and Costa Rica. Locally-held workshops addressed the themes relevant to each region to come up with a national strategy to combat amphibian declines. Certain themes within the ACAP were more relevant to one country than the other and the respective National Action Plans were tailored to reflect this. For instance, over-harvesting is prevalent in Madagascar but is not considered such a pertinent threat in Costa Rica. Conversely, while chytrid fungus has decimated amphibian populations in Central America, preliminary tests on amphibians in Madagascar have turned up negative for the disease. While on the surface this appears like good news for Madagascar, it could also spell potential catastrophe for amphibians if the disease reaches the island. Therefore, although disease is not currently a major threat to the amphibians of Madagascar, lessons from Central America and elsewhere suggest that it would be prudent to be vigilant for the first signs of disease and take necessary precautions to avoid its introduction and spread.

The ACAP provides an overview of the status of amphibians and necessary actions to stem their decline at the time of publishing. Because the situation is constantly changing the ACAP is by necessity a living document and will evolve to reflect these changes. We aim to build upon a growing body of knowledge and enhance communication among stakeholders to streamline efforts to conserve amphibians around the world. The following action steps are designed to aid the integration of ACAP into existing frameworks and new initiatives with a focus on amphibian conservation and research. The cost associated with implementing these actions is detailed in budgets associated with each Chapter and consolidated in the executive summary.

Identifying, Prioritizing and Safeguarding Key Biodiversity Areas

Key Biodiversity Areas, or KBAs, are globally important sites that are large enough or sufficiently interconnected to ensure the persistence of populations of the species for which they are important. A number of steps are outlined in Chapter 1 for the development of a KBA strategy within the greater ACAP. These steps range from identifying, refining and prioritizing KBAs to emergency, short and long-term actions. The following are suggested as a first set of actions:

- Set up a KBA committee/working group to oversee and coordinate the identification of KBAs globally.
- Rank sites so as to prioritize the safeguarding of known amphibian AZE sites first. AZE sites are a particularly sensitive subset of KBAs known to contain the last remaining population of a Critically Endangered or Endangered species.
- Encourage site investigations of historical locations where species are assessed as Critically Endangered (Possibly Extinct).
- Encourage field expeditions to little known and under-sampled sites.

Once a potential site has been identified for protection, it is advisable to conduct a preliminary assessment of the site in question. Such an assessment may include:

- The identification of parties with control over land and resource use in the near vicinity and characterization of their influence over impacts on the site. Such parties might include tenured and untenured landowners, resource and land managers, resource users, government agencies, and concession holders.
- A description of use of resources, local and regional demands, and projected evolution of resource use trends.
- Analyses of relevant land and resource use policies and development plans affecting the governance of the site.
- Analyses of the current legal framework governing the site and its practical effectiveness (if laws are in fact observed).
- The evaluation of existing infrastructure and/or facilities.

The information derived from preliminary assessments can then be used towards the proposal of specific conservation action at KBAs. Once priority KBAs have been assessed and a more thorough understanding of the complexities at specific sites is obtained, the next step would be to initiate actions that will be conducive to safeguarding specific KBAs. These actions would typically include the following:

- Secure core areas for KBAs (proposing and establishing protected areas, negotiating land concessions, purchasing land, seeking community stewardship, negotiations with private landowners).
- Where there are already efforts to safeguard KBAs in place through other projects, seek association with the projects' executors in order to maximize efforts and resource use.
- In-country capacity building through national or regional training programs.
- Develop a set of measurable indicators to monitor KBAs.
- Include an education outreach component targeting schools and local communities at local and national levels.
- Launch a publicity campaign at national levels, seeking active media involvement.

It is important to ensure that eventually all KBAs are safeguarded. Our efforts must begin with an evaluation of the state of the biodiversity for which the site was identified as a KBA. Subsequently, we can establish measurable and spatially explicit conservation parameters for the site. To assess pressures, we must consider both direct pressures that drive species and habitat loss as well as conditions that prevent an effective response to these pressures.

Priority science gaps and future focus

These are some of the questions and gaps that have to be taken into consideration for effective conservation of key sites:

- Increase accuracy and fine-tuning of KBA delimitations.
- Develop an adaptive strategy to deal with fluctuating populations, habitat fragmentation and shifting distributions.
- Integrate related research (disease, climate change, ecotoxicology) into identification of KBAs as there must be connectivity and communication between these different sub-disciplines.

- Engage all stakeholders that could potentially contribute to amphibian conservation, including climatologists, meteorologists, mathematical modelers, environmental engineers, international lawyers, educators, politicians and public relations experts.

Freshwater resources and associated terrestrial landscapes

Threats to amphibians involve alterations to both freshwater and terrestrial habitats. Chapter 2 addresses these threats and outlines steps to protecting critical aquatic and terrestrial amphibian habitat. Specific actions include:

Securing existing habitat

- Research: Identify key habitat requirements (aquatic & terrestrial).
- Education: Develop and implement curriculum for primary through secondary/high school students; outreach program for general public.
- Policy: Develop educational outreach program for policy makers.
- Management: Provide habitat management guidelines for amphibian habitat to land managers and land owners.

Preventing future habitat loss

- Research: Quantify effects of anthropogenic perturbations on amphibian populations.
- Education: Develop and implement curriculum for primary through secondary/high school students and outreach program for general public on how individual behaviors can be modified to improve watershed health.
- Policy: Educate policy makers on impacts of industry, land use, and agriculture on local watershed health, and long-term consequences for human health and local economies.
- Management: Provide habitat management guidelines to minimize future habitat loss for land managers and land owners.
- Research: Identify restoration methods that improve amphibian habitat and population size (adaptive management).

Restoring disturbed or compromised habitats

- Education: Develop and implement educational curriculum to the public to demonstrate value of habitat restoration and healthy amphibian communities.
- Policy: Educate policy makers on the value of amphibian habitat restoration to human and ecosystem health.
- Management: Collaborate with land managers and property owners to develop effective restoration practices.

Climate Change, Biodiversity Loss, and Amphibian Declines

To address the impacts of climate change on amphibian communities addressed in Chapter 3, research is needed to:

- Understand how climate change affects ecosystems and amphibians. Focus should be on changes in disease dynamics and the underlying mechanisms.
- In light of these mechanisms, identify key elements of climate and quantify the relevant changes.
- Develop a predictive model of amphibian decline patterns based on these mechanisms and observed trends.
- Investigate why climate change impacts are worse today than in the past (e.g., landscape alterations, etc.).
- Examine the context of declines to understand broader implications for biodiversity loss (what are the parallels in other groups).

Going beyond research, conservation actions in relation to climate change and amphibian declines need to:

- Increase public awareness about effects of climate change: create educational/outreach/research centers, web sites, positions in existing institutions.
- Promote changes in energy policy. Amphibian declines are critical in defining "dangerous human interference" in the climate system.
- Support initiatives that increase community resilience and reduce sensitivity to climate change (habitat restoration, corridors, etc.).
- Explore the possibility of manipulating local and micro-scale climate in light of mechanisms identified.

Emerging Infectious Diseases

Chapter 4 outlines action steps relating to the detection and control of a disease which currently poses a threat to amphibian populations in many regions of the world; the fungal disease chytridiomycosis.

Disease detection

Clinical signs of infection with chytrid in wild animals are either not present, or not obvious until close to death. As the infection progresses, animals may become lethargic, may exhibit increased sloughing of the skin, especially of the feet and ventrum in adults, or may sit in a characteristic posture in which the hind legs and drink patch are elevated off the ground to minimize contact with substrate. Many older tadpoles of some species may show malformations of the keratinized mouthparts, but this is not consistent across species, stages, or habitats and the presence of infection needs to be verified with histology, PCR, or microscopy. Because of the lack of grossly visible clinical signs, the pathogen needs to be detected by either microscopy (e.g., standard histopathology of the feet or groin skin) or PCR.

Management

There are no currently available vaccines for chytridiomycosis. The treatment of amphibians in the wild with anti-fungal agents would also be problematic. Therefore, simple population management strategies are the only viable option. These may include capture of wild individuals, treatment with drugs that kill Batrachochytrium dendrobatidis or heat, which can kill the fungus, then breeding in captivity ready for release into an area deemed free of disease. Collecting animals for survival assurance colonies may be timed to move ahead of any direction of epidemic spread. Disinfection of footwear with 10 percent chlorine bleach solution to prevent the spread of diseases by tourists and other people moving into sites with 'at risk' populations has been proposed (www.nwhc.usgs.gov/research/amph_dc/sop_mailing.html).

We encourage projects that implement imaginative approaches to treating animals in the wild, modifying habitats to curtail disease spread (e.g., treating vehicles and people to reduce risk of pathogen dispersal) and other procedures to prevent extinction by infection. One crucial part of the armory is understanding why some species are tolerant (e.g., bullfrogs), able to clear infection and recover (e.g., salamanders), or completely resistant to infection. Other strategies may involve captive breeding to select for resistance to *B. dendrobatidis* and other diseases, or even biological control or release of genetically modified pathogens or frogs, while assessing the ethical and conservation implications of releasing such animals back into the wild. One of the most important strategies to help mitigate the impact of chytridiomycosis is to develop the infrastructure for surveillance and population management at the sites that are likely to be affected by this disease in the future.

Research needs

An ambitious research agenda is required, directed to understanding why some populations and species of amphibians become extinct in some regions, whereas others do not, even when faced with the same emerging disease. This ecological research agenda will include studying persistence of the pathogen, reservoir hosts, mechanisms of spread, interactions with climate change and models of disease dynamics. Crucially, these studies will be targeted to 1) sites where amphibians are undergoing enigmatic declines due to chytridiomycosis, linked with studies of climate change, habitat loss, etc., and 2) sites where *B. dendrobatidis* is present, yet populations of amphibians persist without declines.

Research into disease control is critical. More research into the ecology of *B. dendrobatidis* is also needed, including such basic and critical aspects of its natural history as how and where it survives and how long it can persist in the environment. One of the highest priorities is to determine the means by which *B. dendrobatidis* moves among sites, species, and individuals over local, regional and international scales. Monitoring of the trade in amphibians, testing animals throughout that trade and dealing with the policy implications of trying to block disease in those trades is a key priority. Studies of the ecology of chytridiomycosis and other diseases should include broad surveys of its altitudinal and latitudinal distribution and impact, modeling of amphibian population responses to climate change and how this alters disease dynamics, study of the relationship between its spread and trade in amphibians and other key issues.

Finally, we need to continue to survey museum collections and conduct molecular phylogenetic studies to find out when and where *Batrachochytrium* first emerged or whether its distribution has always been wide, and to survey where it is now, in areas with either declining or stable populations. Systematists are encouraged to work with disease researchers to identify declines consistent with disease and to help sample for disease in collections. Part of this research agenda will be to continue to develop cheaper and more efficient testing methods for biological and environmental samples—products that will benefit reintroduction, disease outbreak investigations, as well as survey programs.

Over-harvesting

The purpose of the ACAP workshop on over-harvesting, which forms the framework for Chapter 5, was to establish a harvest management programme, concentrating on 15 countries that appeared to be the focus of the heaviest levels of harvest. The actions needed to address this threat are broadly grouped into six main areas:

Sustainable use

- Study the feasibility and develop sustainable use projects (when the biology of the species permits this) of common and widespread species with local communities.
- Determine whether to implement a controlled sustainable trade through a trade quota.
- Form alliances and allocate resources for expanding these actions to other places.

Species Action Plans

- Continually identify endangered species threatened by over-harvesting from the information generated from trade monitoring and the GAA dataset.
- Establish conservation action plans for threatened species based on the most updated information.
- Allocate adequate resources for implementation of such plans in collaboration with relevant local bodies and stakeholders.

Trade monitoring

- Establish national networks in priority countries to monitor trade. This will involve gathering import/export statistics, commercial breeding farm data and regular visits to the food, medicinal and pet markets.
- Establish collaboration with TRAFFIC to monitor the International trade and trade in CITES-listed species.
- Provide data directly to the GAA team for assessment and dissemination.

Commercial breeding/raising

- Determine the feasibility of establishing new breeding facilities by using scientific data and business costs.
- Ensure that commercial captive breeding facilities use only species native to their regions to reduce the risk of the spread of disease and invasive exotics.
- Carefully monitor commercial breeding farms for highly valuable species to prevent wild-caught individuals from entering into the trade.
- Establish operational certification systems and allocate resources to explore how to help bring such conditions into place.
- Channel (wherever possible) the benefits generated from commercial captive breeding operations with a proportion of profits returning to conservation in the wild.

Law and enforcement

- Strengthen enforcement of relevant law and regulations should be strengthened through capacity-building and the input of adequate resources to prevent over-harvesting.
- Clarify the authority over the conservation, trade and use of amphibians. Better coordination between government bodies and scientific/conservation organizations is needed for effective enforcement.
- Review national law and regulations to make sure they offer adequate protection to the threatened amphibians.
- Improve bilateral cooperation between countries involved in the cross-border trade to prevent over-harvesting and illegal trade of amphibians.
- List species that are threatened by international trade on the appropriate appendices of CITES so that their trade can be regulated and effectively monitored.

Awareness raising

- Convey the importance of amphibians and the widespread impact of over-harvesting to the general public and those in charge of biodiversity conservation in the priority countries through the local media and publicity campaigns.
- Provide local examples of amphibians that should be used in such campaigns.
- Link the publicity campaign with other themes to give a comprehensive picture of the global crisis of amphibian declines.

Mitigating Impacts of Environmental Contamination on Amphibian Populations

Evidence suggests that contaminants in the presence of other stressors have a strong potential to impact amphibians negatively. Efforts to mitigate these impacts, detailed in Chapter 6, are divided into "emergency," "short-term," and "long-term" actions that would be protective of amphibians and the communities in which they live.

Sites where declines are occurring should be evaluated for environmental contamination that may be present through direct application or movement through air or water; this survey data would help us determine if dangerous levels of contamination are present, which may necessitate emergency clean-up action, and would establish chemicals present to guide pertinent research efforts (e.g., interactive effects of contamination and pathogens). Short-term goals should focus on examining the relationships between declines and potential causes; evidence that contaminants routinely are correlated with declines would offer a "weight of evidence" to support the relationships between declines and contaminants, which would justify regulating contaminant application more rigorously. Long-term goals should focus on experimental studies that lead to cause-and-effect relationships to further influence regulatory standards in ways that have meaningful impacts on organisms, as well as further our understanding of how contaminants are influencing community regulation of amphibian communities.

Captive Programs

Chapter 7 focuses on captive programs, which may be an essential component of integrated amphibian conservation plans to avoid imminent extinction of populations. The traditional zoo/aquarium/garden infrastructure cannot currently accommodate a program on the scale required. A global network of captive breeding programs that are explicitly linked to conservation and research programs—The Amphibian Ark (AArk see www.aamphibianark.org)—has therefore been formed to implement the ex situ component of ACAP. Activities will be implemented in four phases:

Information gathering and emergency collections; preliminary captive operations

Operating in response to recommendations from local biologists, national governments, and the various ACAP research branches, rapid-response teams would travel to sites predicted to suffer catastrophic losses to implement pre-emptive collections of animals that will form the basis of captive programs. A prototype of such a program has been used effectively to rescue the frog fauna of a site in Panama (see www.saveafrog.org).

Establishment of captive operations in the range countries

Central to the long-term success of a captive program is the establishment of captive operations in range countries. Infrastructure for such facilities may be reasonably established with portable, modular units (e.g., modified shipping containers) or by simply adapting local warehouses or houses or local infrastructure such as botanic gardens, university biology departments, industrial or government complexes that are either under-utilized or purpose adapted for the management of amphibian species. Local biologists or citizens must quickly be identified, hired, and trained in basic amphibian husbandry. A steady program of internships in established amphibian facilities in other countries will be critical to maintaining intellectual and practical capacity at range-country facilities. Close contact and communication among all facilities in the network must be maintained by a global supervisory staff. Range-country programs will operate in native languages, and will be aimed to ensure that operative protocols are matched to local conditions, culture, and infrastructure.

Research and long-term maintenance of captive operations

In addition to securing captive colonies in small, modular facilities, back-up populations will be secured in larger, multi-species facilities that provide for efficient care, breeding, and research on many species. These larger facilities may be in the range country and/or in facilities and programs outside the range country. Furthermore, these facilities will provide the capacity and facilities for research and implementation of cryobanking of gametes of threatened species, thereby serving as an additional safeguard for species, populations and specific genetic lineages.

Providing animals for research and reintroduction programs

The captive colonies will produce the animals needed to meet long-term research needs and to provide animals for the ultimate goal of reintroduction to natural habitats.

Priority science gaps for research and future focus

Many of the species in need of urgent implementation of captive programs have never before been maintained in captivity. Thus, most programs will face substantial challenges related to basic husbandry and reproduction at the outset. While these captive colonies will represent a crucial element of the overall survival plan for a particular species, they will simultaneously provide important opportunities to conduct research related to disease susceptibility, management and treatments, reproductive biology, and tolerance of environmental elements related to climate and toxins. For example, while various ACAP groups work to better understand the biology, pathology, and potential to control chytrid fungi, captive programs must work with geneticists and immunologists to research the potential for populations to evolve resistance to the fungal pathogen.

Cryobanking

Our general aim is to establish and sustain an active genome resource bank that can contribute to conserving rare amphibians. To achieve this aim, the immediate research objectives are to:

- determine optimal model species that represent a range of amphibian orders and then, through systematic studies, determine fundamental reproductive strategies for each.
- develop safe, non-invasive methodologies for recovering viable sperm.
- increase our understanding of cryosensitivity of amphibian spermatozoa.
- develop 'field-friendly' sperm cryopreservation technologies and tools for assessing the viability/functionality of thawed sperm.
- establish methods for recovering viable spermatozoa from fresh carcasses.
- demonstrate the biological competence of cryopreserved spermatozoa through the production of healthy offspring.
- conduct the necessary computer modeling required to determine the optimal number of individuals to be banked.
- implement and maintain an inventory and database for effective management of cryopreserved samples.
- Increase scientific capacity in-country through training to routinely allow large-scale and safe collection and cryopreservation of germplasm from free-living and captive amphibians.

Most of the research objectives could be met, at least initially, by scientists working in (or with) zoological collections. These individuals must have a strong commitment to capacity building, especially the training of counterparts in range countries that have high priority species requiring attention. Ideally, studies would begin in North American zoos and, once the models were identified and research colonies developed, studies would begin, preferably with a senior scientist mentoring multiple post-doctoral fellows, graduate or undergraduate students. Some of the trainees eventually must come from range countries where there is an eventual goal to develop research/propagation programs for high priority species. This will require the development of laboratory and *ex situ* breeding facilities.

Reintroductions

The IUCN (1998) guidelines for reintroductions provide a framework for the protocols to be followed for amphibians, but may need modifying in view of species-specific requirements or linkages to other themes within ACAP. Aspects to take into consideration when planning a reintroduction are detailed in Chapter 8 and summarized below.

Selecting species for reintroduction

It is essential that species are carefully appraised for their suitability for reintroduction. The following criteria, which are elaborated in Chapter 8, provide guidance for evaluating whether a species is suitable for reintroduction:

- Status and distribution of species.
- Reversibility of threats.
- Life history.
- Geographical priorities.

Pre-release assessment of the wild populations

The status and distribution of the species will be assessed by a combination of interrogation of existing sources of information (e.g., GAA, local atlases etc.) and field survey. Refinement of existing survey methodologies may be required as an adjunct research activity to allow this. Priority species will be those that have undergone clear contractions in historical range, and which would be unable to re-establish functional populations (or metapopulations) within that range without reintroduction. Introductions to areas outside the historical range will usually be discouraged, although climate change data may suggest that unsuitable areas outside the natural range may become suitable sometime in the future. Equally, restocking (or supplementing) existing populations carries disease and genetic risks (see below) and should not be considered unless numbers have fallen below those required for a minimum viable population and the associated risks have been assessed.

Applied ecological research on life history and habitat requirements

Basic population demographic data on the species will 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 the reintroductions. 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 reintroduction succeeding.

Threat mitigation, habitat management, restoration and creation

The threats leading to the decline or extinction of the species will be evaluated and neutralized following the protocol described by Caughley (1994). It is unlikely that some important threats to amphibians (e.g., climate change, UV-B, etc.) can be neutralized, at least in the short to medium term. In such cases, reintroduction is unlikely to be a sensible option.

Following the assessment of habitat requirements, potential reintroduction sites will be evaluated for management requirements. The program of habitat management will involve maintaining or enhancing existing areas, restoring areas that still exist but have become unsuitable and creation of new habitat where appropriate (or a combination thereof).

Population viability analysis, release protocols, and strategic recovery plan development

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. These targets should then be embraced within a staged planning process, with interim milestones if necessary to monitor progress as the project develops. Knowledge of the life history of the species should be used to determine appropriate targets and time frames for success. EU legislation requires member states to maintain—or restore to—'favorable conservation status' those species of community interest, and this is being used as a generic target in many species recovery programs (although explicit definitions of this term may vary from species to species, and region to region).

The reintroductions will involve the release of eggs, larvae and/or metamorphs, as previous reintroduction programs have shown that using these stages is most likely to lead to success. However, further research is needed on release protocols (e.g., the relative proportions of the different stages, 'soft' vs. 'hard' releases, trade-offs of captive vs. wild stock, applicability of head-starting technologies). The reintroductions will therefore serve as ecological experiments for testing hypotheses concerning these issues, and protocols will be refined accordingly.

An appropriate organizational infrastructure needs to be established to ensure the success of the program. This will invariably require the cooperation of a wide spectrum of stakeholders ranging from local communities to government officials. There may be legal obstacles associated with the release of organisms into the wild that need to be overcome. Effective lines of communication need to be established, language barriers overcome and transparent mechanisms for resolving differences of opinions established.

Risk analysis

The movement of living organisms from one place to another carries various risks. These risks may be genetic, ecological or socio-economic. Genetic risks are associated with the release of maladapted animals into an area. Donor populations will be screened for any potential problems associated will possible maladaptations or inbreeding. This will be combined with a landscape level analysis of the release site to ensure that the released population will not suffer from any genetic problems as a result of habitat isolation in the future. There may also be concern over the release of animals whose taxonomic relationships are unresolved. Linkage with the ACAP Systematics Working Group will be maintained to resolve any issues in this area.

Ecological risks embrace issues associated with the inadvertent transmission of disease or other organisms. Apparently benign organisms may have unforeseen impacts on food chains when transmitted to new environments. Protocols will therefore be in place to minimize the risk of transmission of propagules of potentially invasive species. Comprehensive health screening will be carried out on 1) animals from the donor population (captive or wild); 2) all amphibian species present at the release site. The protocols will follow those established by the ACAP Disease Working Group (See Chapter 4). Socio-economic risks are associated with impacts on the livelihoods of local people. If the reintroduction results in the exclusion of people from traditional areas or ecological impacts that impact on agriculture or other income-generating activities, there may be ramifications for its likely success. Surveys of attitudes towards the reintroduction within local communities will therefore be carried out and any conflicts of interest resolved.

Post-release monitoring

Many amphibian species have cryptic life styles that render them extremely difficult to monitor. Consequently, research on the refinement of monitoring protocols will inform the design of post-release monitoring. Equally, the longer the generation time of the species the longer the timeframe needed for establishing 'success'. In order to demonstrate whether the 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.

Systematics

Chapter 10 focuses on Systematics and Conservation and proposes the following activities:

- Naming species (1000 spp. over the next five years).
- Training in-country students and auxiliary personnel (e.g, park guards, etc.) and support for in-country experts:
- Short term visitation of experts and students to research centers.
- Systematic workshops for students and young professionals.
- Grants to pursue graduate school or postdoctoral work in systematics, this grant could be for in-country or foreign institutions.
- Amphibians field surveys in poorly known areas and areas that have not being survey in the last decade.
- Genetic bar coding (1000 spp.).
- Frozen tissue bank of all taxa for molecular analysis and forcell banks.
- Evaluation of Critically Endangered and Endangered species in a phylogenetic analysis to prioritize taxa for conservation.
- Evaluation of Data Deficient taxa for conservation.
- Publication of field guides, in local languages.
- Establishment, improvement, and maintaining local collections.

Bioresource Banking

Genome Resource Banks (GRBs), which form the focus of Chapter 11, can provide vital materials, such as high quality DNA, cellular RNAs and cell fractions, for research as well as enhancing reproduction and rescuing genetic variation that would otherwise be lost. Collection of research samples most feasibly could come from salvaged specimens and entail no harm to wild or captive populations. The highest quality resource that might be obtained from individuals at post-mortem examination would likely be viable cell cultures. Because only a small literature exists describing establishment and freezing of cell cultures from amphibians, priority action in bioresource banking will need to concentrate effort on productive collaboration among field biologists, captive breeding efforts, pathologists and those involved in cell culture and cell banking. The Frozen Zoo at the San Diego Zoo's center for Conservation and Research for Endangered Species (CRES), which demonstrates a successful history in mammalian, avian and reptile cell culture, is suitable and willing to immediately undertake efforts to establish cell cultures for the first time in accordance with the ACAP.

Chapter 1

Designing a Network of Conservation Sites for Amphibians— Key Biodiversity Areas

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1.1 Introduction

Results from the Global Amphibian Assessment (GAA: www.globalamphibians.org) provide a much-needed baseline for global amphibian conservation. These data can be used to design strategies to conserve the world's rapidly declining amphibian populations. For nine out of every ten amphibian species that are classified as threatened, habitat loss is a key threat (Baillie *et al.* 2004). It is therefore critical to identify and safeguard those sites where highly threatened amphibians occur in the short term. Strengthening and expanding systems of protected areas (PAs), private reserves, and other conservation sites containing critical habitat for amphibians must be the top priority for conservationists (Young *et al.* 2004) as we seek to maximize the return from conservation investments.

Current PA systems, however, are incomplete and do not adequately capture global biodiversity; often leaving out threatened species for which site conservation is more urgently needed (Pressey 1994). Although the current network covers 11.5% of the terrestrial land surface, global assessments reveal large gaps in the existing network of protected areas in almost all regions, particularly in the tropics (Brooks *et al.* 2004; Ferrier *et al.* 2004). Filling these gaps requires the establishment of explicit, measurable, and repeatable targets for biodiversity conservation (Rodrigues *et al.* 2004a).

Global assessments are extremely important for audiences operating at the same scale, including bilateral and multilateral organizations such as the World Bank, international policies such as the Convention on Biological Diversity (CBD), and nongovernmental organizations with a global scope. However, this kind of global assessment is far from providing an accurate picture of the coverage, e*t al.* one the viability and effectiveness, of protected areas on a country-by-country or region-by-region scale (Brooks *et al.* 2004). Such assessment needs to be driven from sub-global scales to incorporate the complexities of fine-scale data, but it also needs to follow international standards and criteria if it is to be globally comparable. The concept of Key Biodiversity Areas (KBAs) is a tool for achieving this (Eken *et al.* 2004). Site-by-site assessment of the management effectiveness of such areas in safeguarding the biodiversity for which they are important will be necessary to produce a truly accurate analysis.

1.2 Key Biodiversity Areas

Key Biodiversity Areas, or KBAs, are globally important sites that are large enough or sufficiently interconnected to ensure the persistence of populations of the species for which they are important (Eken *et al.* 2004). The KBA approach, based on the concepts of threat and irreplaceability that are central to systematic conservation planning (Margules & Pressey 2000), incorporates detailed information on different species' conservation needs and on the adequacy of particular areas for the conservation of each species. In proposing a universal standard set of thresholds, it is possible to minimize subjectivity in the selection of globally important sites and to ensure repeatability in the application of KBA criteria. The application of these criteria should be straightforward, so that they may be consistently applied across different biogeographic regions and taxonomic groups; at either national or regional levels.

The focus of KBAs is on those species most vulnerable to extinction: globally threatened species[3] and geographically concentrated species. Information on the distribution and needs of these species is used to define those sites that will be critical to their survival. A proposed approach to prioritization among KBAs incorporates considerations of irreplaceability, threat, and cost.

KBAs are defined and mapped using biological and geophysical data. The boundaries are further refined using sociopolitical data, such as existing PAs, land tenure and other management units. Information is compiled for each site on threats, protected status, and key conservation actions. The KBA selection process uses four criteria, based on the presence of species for which site-scale conservation is appropriate: (1) globally threatened species, (2) restricted-range species, (3) congregations of species that concentrate at particular sites during some stage in their life cycle, and (4) bioregionally restricted assemblages (Eken *et al.* 2004). A KBA network defined according to the presence of these species would, therefore, be expected to embrace all sites that play a critical role in maintaining the global population of a particular species (Eken *et al.* 2004). All four criteria have been applied to identify KBAs for one higher level taxonomic group (birds) for over 20 years—by BirdLife International, through their Important Bird Areas (IBA) program—and the effectiveness of this approach in identifying site conservation priorities has been validated by extensive research (BirdLife International 2004).

A particularly sensitive subset of KBAs, are those sites known to contain the last remaining population of a Critically Endangered or Endangered species (Ricketts *et al.* 2005). Such sites, identified by the Alliance for Zero Extinction (AZE; www.zeroextinction.org), a joint partnership of more than 60 biodiversity conservation organizations, form the highest priority subset of KBAs. Currently, about 260 AZE sites are triggered by amphibians (representing some 380 species). It should be stressed at this point that for many of these species, the nature of threat is such that habitat protection alone may not be sufficient to ensure the long-term, or even short-term persistence of the species, particularly not in the face of insidious threats such as disease (specifically chytridiomycosis) and climate change. Nonetheless, habitat protection must be a necessary in situ conservation action implemented in tandem with complementary ex situ measures (such as captive breeding).

1.3 Key Conservation Actions

There are a number of steps that must be taken towards the development of a KBA strategy within the greater ACAP. These steps range from identifying, refining and prioritizing KBAs to emergency, short and long-term actions.

[3] Species listed as Critically Endangered (CR), Endangered (EN) or Vulnerable (VU) on the IUCN Red List of Threatened Species (www.iucnredlist.org)

1.3.1 Initial Actions—Setup and Framework of KBAs

As a first set of actions required to move forward with the KBA component of ACAP, we suggest the following:

- Set up a KBA committee/working group to oversee and coordinate the identification of KBAs globally.
- Rank sites so as to prioritize the safeguarding of known amphibian AZE sites first. While all AZE sites/species are important and absolutely urgent, in the context of site-scale conservation, the most immediate priorities will be those sites/species for which the overwhelming threat is loss of native habitat and for which the most obvious conservation action involves habitat protection, maintenance, or restoration. For example, the Massif de la Hotte in Haiti is the only known site for no fewer than 13 Critically Endangered or Endangered amphibians, and the overwhelming threat at this site is not one of disease but rather ongoing habitat loss and degradation. Such sites (i.e., those where site-based threat and habitat loss are high) should represent the most immediate priorities for safeguarding.
- Continue refining the KBA analysis when new information becomes available.
- Encourage site investigations of historical locations where species are assessed as Critically Endangered (Possibly Extinct).
- Encourage field expeditions to little known and under-sampled sites.

1.3.2 Preliminary site conservation assessment of KBAs

Site and resource management planning tends to be very complex and cannot be entered into lightly. Once a potential site has been identified, it is advisable to conduct a preliminary assessment of the site in question. Such an assessment may include:

- The identification of parties with control over land and resource use in the near vicinity and characterization of their influence over impacts on the site. Such parties might include tenured and untenured landowners, resource and land managers, resource users, government agencies, and concession holders
- A description of use of resources, local and regional demands, and projected evolution of resource use trends
- Analyses of relevant land and resource use policies and development plans affecting the governance of the site
- Analyses of the current legal framework governing the site and its practical effectiveness (if laws are in fact observed)
- The evaluation of existing infrastructure and/or facilities.

The information derived from preliminary assessments can then be used towards the proposal of specific conservation action at KBAs.

1.3.3 Implementation of conservation actions at KBAs

Once priority KBAs have been assessed and a more thorough understanding of the complexities at specific sites is obtained, the next step would be to initiate actions that will be conducive to safeguarding specific KBAs. These actions would typically include the following:

- Securing core areas for KBAs (proposing and establishing protected areas, negotiating land concessions, purchasing land, seeking community stewardship, negotiations with private landowners).
- Where there are already efforts to safeguard KBAs in place through other projects, seek association with the projects' executors in order to maximize efforts and resource use.
- In-country capacity building through national or regional training programs.
- Develop a set of measurable indicators to monitor KBAs.
- Include an education outreach component targeting schools and local communities at local and national levels.
- Launch a publicity campaign at national levels, seeking active media involvement.

It is worth mentioning that, with regards to protected areas, which are one of the means of safeguarding KBAs, the Convention on Biological Diversity program of work on protected areas contains a worthy target to address the funding gap: "By 2008, sufficient financial, technical and other resources to meet the costs to effectively implement and manage national and regional systems of protected areas are secured, including both from national and international sources, particularly to support the needs of developing countries and countries with economies in transition and small island developing States" (CBD 2004).

1.4 Challenges and Opportunities

1.4.1 Challenges

Attempting to implement KBAs as part of a global ACAP can be at times daunting, if only because of the scope and magnitude of the task. Considering those practical aspects involved, we identify the main challenges as follows:

- Finding funding sources and sequestering funds to implement ACAP—this is likely to be one of the most challenging points, in relation not only to KBAs, but also to other thematic areas of ACAP.
- Balancing the needs of multiple stakeholders—typically a number of different stakeholders will be involved in the process of KBA delimitation and conservation of sites. Many stakeholders will have different agendas and vested interests and it is also probable that for several actors, interest levels will have to be raised with a hard-sell case for amphibians.
- There is the very real potential for existence of factors external to the sites in question that can adversely affect the populations within the sites themselves (e.g. disease, pollution, introduced species, etc.), and identifying and containing these factors will not only be a challenge but also a priority to ensure that these actions occur in parallel with on-going work to safeguard KBAs.

1.4.2 Opportunities

There are a number of opportunities and advantages that come with the development and conservation of KBAs, some of which can act as catalysts towards other conservation actions, such as:

- The establishment of an emergency action element, to identify and safeguard the 120 KBAs most at risk of being lost.
- Some of these prospective KBAs are already protected, so it would not be necessary to start from scratch (i.e. potential for leveraging with other existing NGOs or existing projects).
- The protection of sites for amphibians is likely to include protection of other flora and fauna, or on a community assemblage, having thus added benefits in the form of maintenance of ecosystem balance.
- People interested in conserving sites may come from different constituencies, increasing the potential for further outreach, communication and collaboration, and diversity of stakeholders.

1.4.3 Logistics, infrastructure, policy and capacity needs

It is important to ensure that eventually all KBAs are safeguarded—whether that is through government designation, formal agreement with the landholder, or other mechanisms—and effectively managed to conserve the biodiversity they contain. Our efforts must begin with an evaluation of the state of the biodiversity for which the site was identified as a KBA, which involves consolidating key data on, and assessing the status of, the globally threatened and geographically concentrated species for which a site has been identified as globally important for biodiversity conservation. Subsequently, we can establish measurable and spatially explicit conservation parameters for the site. To assess pressures, we must consider both direct pressures that drive species and habitat loss as well as conditions that prevent an effective response to these pressures. We have identified the following needs to bring forward the identification, assessment, and conservation of KBAs.

1.4.4 Needs for Initial Actions

- Identify those qualified specialists who would be willing to be a part of the KBA committee/working group to oversee and coordinate the identification and prioritization of KBAs globally.
- Establish a KBA—identification seed grant system whereby field research of historical locations and little-known, under sampled sites, are emphasized.
- Hire qualified personnel to carry out coordination and implementation of seed grant system and reporting back to KBA working group.
- Identify funding sources, proposal-writing and lobbying to access funds to cover priority actions.

1.4.5 Needs for a preliminary assessment of prospective KBAs

- Access to records and information that will allow for the identification of parties with control over land and resource use in the near vicinity, communication with these parties and their cooperation to realistically characterize their influence over impacts on the site.
- Establish communication (and potentially future partnerships) with parties with influence over the conservation of the protected area and the broader landscape, and cooperation of these parties to characterize their influence and impact on the site.
- Access to records and information (e.g. through surveys) on use of resources, local and regional demands.
- Access to current government policies regarding land and resource use as well as access to development plans affecting the governance of the site.
- Access to current legal framework governing the site; compilation of information on practical effectiveness (i.e., through surveys).
- Access to site and vicinity to conduct evaluation of existing infrastructure and/or facilities and identification of relevant parties.
- Increased human resources (trained personnel) to conduct assessments through seed grants.

1.4.6 Needs for the implementation of conservation action in KBAs

- Availability of funds, negotiating ability and leverage to secure core areas for KBAs.
- Developing and strengthening partnerships where there are already existing projects that require some/full implementation of conservation in KBAs.
- Establishing a seed grant fund to encourage in-country students and researchers to conduct research in KBAs.
- Availability of funds and qualified instructors for in-country capacity-building through national or regional training programs.
- Establishing a permanent emergency fund for critical site-based actions.
- Elaborating a conservation strategy and site management plan for each priority KBA.
- Lobbying to engage governments in national conservation efforts.
- Building partnerships with local NGOs and grassroots organizations.
- Planning and developing a conservation education program targeting schools and local communities at national levels.
- Producing publicity outputs (brochures, posters) and involving the media in a publicity campaign.

1.4.7 Priority science gaps and future focus

There are a number of fronts that need to be developed and issues that need to be addressed simultaneously in order to increase the efficiency of ACAP as a whole. With regards to KBAs, these are some of the questions and gaps that have to be taken into consideration for effective conservation of key sites:

- Increase accuracy and fine-tuning of KBA delimitations.
- Develop an adaptive strategy to deal with fluctuating populations, habitat fragmentation and shifting distributions.
- Integrate related research (disease, climate change, ecotoxicology) into identification of KBAs as there must be connectivity and communication between these different sub-disciplines.
- Engage all stakeholders that could potentially contribute to amphibian conservation. Although its scope is integrated in the field, as it seeks to connect the different thematic areas, a wealth of expertise outside of the amphibian experts' community will be needed to address the problem of the amphibian extinction crisis in an efficient manner. This will require the support of climatologists, meteorologists, mathematical modelers, environmental engineers, international lawyers, educators, politicians and public relations experts, to mention a few. In order to increase the efficiency of ACAP, additional expertise in a diversity of fields should be enlisted. Although it would appear that this would likely increase costs, it would probably be more cost-efficient in the long run by decreasing the time span needed to carry out specific actions that would otherwise take longer if performed in isolation, and perhaps not be as efficient as a multidisciplinary approach.

1.5 Budget

Bruner and colleagues (2004), recently published an article where they draw on published cost studies, working sessions on protected area costs from the Fifth World Parks Congress (WPC) in 2003, and post-WPC analyses to quantify the funding shortfall for terrestrial protected areas across developing countries and to assess necessary actions to close the gap. Their study indicates that the costs of establishing and managing an expanded protected-area system (approximately 3.5 million km^2) would total at least \$4 billion per year over the next decade, an amount that far exceeds current spending, but is well within the reach of the international community. These findings indicate the need for rapid action to mobilize significant new resources for the developing world's protected areas. In particular, this will require (a) the use of a range of tools to generate funds and improve efficiency of management; (b) greater precision and better communication of the costs and benefits of protected areas, both locally and globally; and (c) increased, stable support from developed countries for on-the-ground management of protected area systems in developing countries.

There is a diversity of ways to address conservation of KBAs (land management concessions, community stewardship, designation of protected areas, land purchase) so that the development of PAs is not the only means by which KBAs can be secured. This diversity in KBA conservation strategies is also reflected in the global budget, coming to much more manageable costs than those estimated by Bruner *et al.* (2004). The following global budget plan, constructed on estimates for a period of five years, is proposed:

Designation costs are difficult to estimate because they are highly variable, ranging from zero (when governments allocate uninhabited public lands) to the full cost of land purchase. However, we believe that, taking these variations into consideration, costs may even out across the board as the cost of land purchase in one area may be offset by the designation of a protected area or community stewardship in another.

Cost	US$
Mechanism to update KBA identification	1,000,000
Field surveys (seed grant system)	5,000,000
In-country capacity building	4,000,000
Kickstart fund*	60,000,000
Emergency fund for critical site based actions	20,000,000
Sustainability fund to ensure future protection of sites	30,000,000
Total	120,000,000

*US$ 500,000 per site: buying or securing core area, paperwork, infrastructure, basic staffing for 5 years, etc. Projection of 120 sites effectively conserved (removal of the threats that we are concerned about).

Chapter 2

Freshwater Resources and Associated Terrestrial Landscapes

M. Lannoo, C. Funk, M. Gadd, T. Halliday and J. Mitchell

2.1 Introduction

Most of the world's amphibian species exhibit a complex life history, with aquatic egg and larval stages followed by a rapid metamorphosis to a terrestrial adult stage. Some amphibian species are completely aquatic, with eggs, larval, and adult stages taking place either in the same water system (rivers, lakes) or in associated water systems (with short migrations overland). Still other amphibian species forgo the larval stage altogether and complete their life cycle on land, in terrestrial ecosystems. Most people associate amphibians with freshwater habitats, particularly ponds, and most active conservation efforts are aimed at the protection of such habitats. For most species that use freshwater habitats only to breed, the time they and their larvae spend in water represents only a small proportion of their life history. On land, amphibians are secretive and widely dispersed and relatively little is known about their natural history in terrestrial habitats. As a result, techniques to conserve amphibians in terrestrial habitats are poorly developed.

A number of recent studies that have compared the ecology of species that have declined dramatically with those that have not, have identified high altitude as a significant 'risk factor' for amphibians (Declining Amphibian Database; Hero and Morrison 2004; Lips *et al.* 2003). This effect is rather surprising, because upland habitats are generally not as affected by habitat change (e.g., agriculture) as lowland habitats, and for many of the declines that occur at higher altitudes, the immediate cause is not obvious.

It follows that if amphibians are to persevere, it is critical, not only that high quality aquatic ecosystems persist, but also that associated terrestrial habitat is protected. The degradation of either ecosystem type derails amphibian life cycles and affected populations become compromised, perhaps extirpated. It is particularly important that upland habitats, aquatic and terrestrial, be protected.

A majority of the world's 5,883 species (AmphibiaWeb, 9 Sept., '05) are found in tropical and low temperate ecosystems (see Fig. 1 and discussion below). Within these regions, direct developing (terrestrial) amphibians can be found almost anywhere on land except at very high altitudes. Amphibians with complex life histories are typically found within the vicinity of sources of freshwater that can be used for breeding. While several studies have attempted to document the distance adult amphibians move from their breeding sites, David Green (pers. comm.) has observed that the maximum distance noted by these studies is correlated with the geographic scope of the study. Our knowledge of the dispersal of amphibians across land is very poor and requires major research input, not least because understanding this aspect of amphibian ecology is vital for effective conservation.

2.2 Ecosystems

Aquatic ecosystems used by amphibians are typically freshwater, although some species breed in brackish water. Lentic freshwater ecosystems that support amphibians range from seasonal wetlands, through semi-permanent and permanent wetlands, to lakes and ponds. Lotic freshwater ecosystems that support amphibians range from seeps and springs, through small and large streams, to rivers. As a general rule, fishes exclude amphibians, therefore ecosystems with fewer fishes provide habitat for more amphibian species: seasonal wetlands support more species than lakes; seeps and springs support more species than rivers. Upland ecosystems are not only important to amphibians by providing habitats, but also human activities at higher watershed levels affect both terrestrial and aquatic habitat quality at lower levels.

2.3 Facts About Freshwater (Lean and Hinrichsen 1992; AAAS 2000)

- Less than 3% of the earth's surface is composed of freshwater.
- More than 75% of this is locked up (although likely not for long) as polar ice.
- 98% of the remaining freshwater lies underground.
- Therefore, only about 0.01% of the world's total freshwater is readily available to terrestrial life.
- Freshwater is unevenly distributed throughout the world, e.g., Canada has 30 times as much freshwater available to each of its citizens as China
- Freshwater is being contaminated by saltwater influxes, human waste and other byproducts of human use (e.g., endocrine disruptors, acid rain), as well as agricultural fertilizers and pesticides.
- Since 1950, the number of people on earth has increased from 2.5 to 6.5 billion, and the per capita use of freshwater has tripled.
- More than 60% of all freshwater used in the world is diverted for irrigating crops.

2.4 Facts About Upland Habitat (Lean and Hinrichsen 1992; AAAS 2000)

- Forests are the planet's largest reservoir of biological diversity, containing an estimated half of all the world's plant and animal species.
- Only about 50% (750 million out of 1.5 billion hectares) of historic mature tropical forests still stand.
- Tropical deforestation is increasing and is currently between 16.4 and 20.4 million hectares/yr.
- Temperate deforestation has been extensive (i.e., only 1.5% of Britain's original forest remains; Poland's Bialoweiza National Park contains the last major ancient forest in Central Europe).
- Logged temperate forests are often replanted as single-species plantations.
- Since the 1970s, large tracts of temperate forests have died.
- Grasslands have great biological value, being the original home of wheat, barley, millet and sorghum, but soils grow slowly and salts tend to build up.
- Since 1700, 560 million hectares of grassland and pasture have been converted to agricultural usage (Ramankutty and Foley 1999).
- Desertification of grassland and pasture threatens about 33% of the world's land surface.

2.5 How Does this Relate to Amphibians?

Amphibians are not distributed uniformly across the surface of the earth (Fig. 1). Except for the few species that live in brackish waters, amphibians occur exclusively in terrestrial or freshwater ecosystems. For any given longitude, species richness is higher near the equator, and lower towards the poles. The highest amphibian richness values occur in the tropical rainforests and moist tropical forests of Central America, South America, Equatorial Africa, and Eastern and Southeastern Asia. Developed regions such as North America, Europe, and Australia tend to have considerably fewer species.

Threats to amphibians involve alterations to both freshwater and terrestrial habitats. Rates of conversion of native vegetation to agriculture have been increasing (Fig. 2), including percent change in agricultural production (Fig. 2A), cropland area (Fig. 2B), and amount of permanent cropland (Fig. 2C).

There has been an increase in the area of land that is irrigated (Fig. 3). We noted previously that over 60% of the freshwater used by humans is water diverted for irrigation.

Forest cover is decreasing globally. These changes include decreases in natural forests (includes closed forests and open forests with at least 10% tree cover; Fig. 4A), decreases in closed canopy forest (Fig. 4b), and decreases in all forest types (natural, managed, etc.; Fig. 4C.).

Figure 5 indicates locations of larger wetlands worldwide, and Figure 6 shows wetlands of international importance, as recognized through the Ramsar Convention agreement (Navid, 1989). Tropical ecoregions, which provide important habitat for a rich diversity of amphibian fauna, are not well represented in the distribution of Ramsar wetlands. Nor are the very numerous ponds and other small freshwater habitats that exist on Earth; despite their small size, such habitats support a high diversity of plants and animals, including amphibians.

Composite rates of population-, agriculture-, and forest-related habitat change are indicated in Figure 7. This map shows the relative magnitude of the rates at which the landscape is being altered in ways that are likely to be detrimental to amphibian habitat. When this composite map of landscape change is compared with the map of amphibian richness (Fig. 8), a disturbing pattern emerges. Throughout much of the world, particularly Central America, northern and eastern South America, western sub-Saharan Africa, Madagascar, eastern India, Southeast Asia, and southern coastal regions of Australia, changes in land cover and land management correspond in an alarming way with regions of high amphibian richness.

2.6 The Conservation And Restoration Of Freshwater and Upland Systems Important to Amphibian Populations

Amphibians are often said to be "canaries in the coal mine" or sensitive indicators of environmental health. The implication here is that ecosystems have been compromised and amphibian populations are telling us this. The reverse, however, is also true. Diseases, such as chytrid fungus, target amphibians that occupy otherwise healthy ecosystems, and this loss of amphibians compromises ecosystems. Attempts to reverse amphibian declines must take both of these factors into account.

The first step towards reversing amphibian declines is to provide the high quality aquatic and terrestrial habitats that amphibians require. In some places these habitats exist, in others they once did but do not anymore. At least some of these latter areas should be the targets of restoration efforts, especially where associated amphibian species are in decline. The good news here is that these ecosystem restoration efforts will be required even in the absence of any primary consideration of amphibians. While oil is perceived to be the current limiting resource, in the future freshwater is likely to be the world's limiting resource. Policies promoting clean freshwater and the protection of sources of clean water through watershed management will undoubtedly benefit remaining amphibian populations.

American actor/comedian W. C. Fields (1940) is reputed to have said that he did not drink water because fish make love in it. He missed the point. If fish, and amphibians, can breed successfully in freshwater, it assures us that it is fit for us to drink.

2.7 Amphibians, Genetics and Landscapes

While our knowledge concerning the terrestrial lives of amphibians is deficient in many ways, we do know that some species can move considerable distances on land and colonize newly created aquatic breeding habitats very quickly. However, they can only do this from existing, established populations and so, if conservation efforts are to be effective, we need to improve knowledge about the movement of amphibians between breeding sites. Dispersal is a key consideration in metapopulation models of amphibians, which emphasize the importance of maintaining networks of breeding sites connected by suitable terrestrial habitat. A number of recent studies have looked at metapopulations within landscapes to determine the effects of geographical distance and habitat type on amphibian dispersal between breeding sites (Lannoo 1998; Semlitsch 1998, 2000). Such studies are most revealing when combined with genetic analyses which determine the 'genetic distance' between sites, that is, how isolated genetically adjacent sites are from one another. It is important that future studies of the relationship between amphibian abundance and terrestrial habitat are informed by the findings from the relatively new disciplines of landscape genetics and conservation genetics.

A study of the European frog *Rana latastei* (Garner *et al.* 2003) has looked at the genetic consequences of breeding site isolation, comparing ponds at the edge of the species' range, with those near the middle. Outlying, more isolated ponds show lower genetic variation. Significantly, animals from such ponds were more susceptible to infectious disease than animals from the core of the range. There is thus a further link that must be maintained, between landscape genetics and infectious disease.

2.8 Habitat Change

Many amphibian habitats are not stable over time, being subject to long-term changes, two of which are of particular importance in the conservation of amphibians: climate change, and ecological succession. Climate change alters temperature and rainfall patterns and thus has huge potential to negatively affect amphibian populations (See Chapter 3). A number of studies have already shown that amphibians are breeding earlier in the year than they were 20 years ago; whether this is beneficial or harmful to their long-term survival is not yet clear. An important implication of climate change is that habitat that is optimal for a particular species may move laterally across the landscape (e.g., many wild plants in the UK have shifted their ranges northwards) or, as in the case of montane habitats such as that at Monteverde, Costa Rica, shrink and eventually disappear altogether. It is important therefore that workers interested in landscape aspects of amphibian conservation maintain close contact with those working on climate change.

Many amphibian breeding habitats are subject to ecological succession. Permanent ponds are typically not permanent, but, if left unaltered, become overgrown and eventually fill in. The active conservation of amphibians will require a great deal of work, much of it experimental, to determine the best ways to manage amphibian habitats over long-periods to offset the effects of succession.

2.9 Actions

We must identify and outline steps to protect critical aquatic and terrestrial amphibian habitat. We identify three primary spatial scales on which to work: 1) continental/ecoregion, 2) watershed (focus), and 3) site levels, as well as the temporal scale, which must be addressed in order to preserve natural successional processes or to restore successional processes in areas that have been altered. Landscape issues should identify and protect the integrity of ecosystems at each of these spatial and temporal scales.

Specific priorities, threats and actions will need to be addressed with respect to the following life history variations: 1) species with a complex life history; 2) fully aquatic species; and 3) species with terrestrial development.

Specific actions to stem the decline of amphibians must directly include:

2.9.1 Securing existing habitat

a. Research: Identify key habitat requirements (aquatic & terrestrial) (budgetted in the KBA chapter).
b. Education: Develop and implement curriculum for primary throughsecondary/high school students; outreach program for general public ($100K per ecoregion per year x 40 ecoregions = $4 million per year).
c. Policy: Develop educational outreach program for policy makers ($3 million per year).
d. Management: Provide habitat management guidelines for amphibian habitat to land managers and land owners ($50K per ecoregion per year x 40 ecoregions = $2 million per year).

2.9.2 Preventing future habitat loss (water use/looking ahead)

e. Research: Quantify effects of anthropogenic perturbations on amphibian populations ($200K per ecoregion per year x 40 ecoregions = $8 million per year).
f. Education: Develop and implement curriculum for primary throughsecondary/high school students and outreach program for general public on how individual behaviors can be modified to improve watershed health (included in 1b).
g. Policy: Educate policy makers on impacts of industry, land use, and agriculture on local watershed health, and long-term consequences for human health and local economies (included in 1c).
h. Management: Provide habitat management guidelines to minimize future habitat loss for land managers and land owners (included in 1d).
i. Research: Identify restoration methods that improve amphibian habitat and population size (adaptive management) ($100K per ecoregion per year x 40 ecoregions = $4 million per year).

2.9.3 Restoring disturbed or compromised habitats

j. Education: Develop and implement educational curriculum to the public to demonstrate value of habitat restoration and healthy amphibian communities (included in 1b).
k. Policy: Educate policy makers on the value of amphibian habitat restoration to human and ecosystem health (included in 1c).
l. Management: Collaborate with land managers and property owners to develop effective restoration practices ($100K per ecoregion per year x 40 ecoregions = $4 million per year).

2.10 Budget

Total Annual Budget = $25 million per year

Total 5-year Budget = $125 million

2.11 List of Figures

(*Editors Note:* Figures accompanying this chapter may found in the online version of ACAP: www.amphibians.org)

Figure 1. Amphibian species richness. The map legend depicts classes of species richness, where each successive class represents twice the number of species as the previous class. Because of large differences in the availability of amphibian distribution information from around the world, species richness may be over-represented in some areas, and under-represented in others (from Gallant *et al.*, *submitted*).

Figure 2. Agricultural changes from 1961 through 1998. A) Percent change in agriculture production. Many parts of the world experienced increased production, but nearly all of the ecoregions in southern Africa had decreased levels of production. Patterns of increase often followed ecoregion boundaries within continents, but similar ecoregion types across continents had different characteristics of change. B) Changes in agricultural production sometimes contrasted with patterns of change in cropland area. C) Amount of permanent cropland (i.e., land supporting crops that persist after harvest, such as orchards and plantations) increased throughout most of the world's agricultural areas.

Figure 3. Changes in forest cover from 1990 to 1995. A) Percent change in natural forests (includes closed forests and open forests with at least 10% tree cover). B) Changes in closed canopy forest shown as a percent of original closed forest cover. C) Percent change for all forest types (natural, managed, etc.).

Figure 4. Percent change in irrigated lands between 1961 and 1998.

Figure 5. Wetland distribution worldwide as recognized by the US Soil and Water Conservation Service Natural Resources Conservation Service, compiled by FAO-UNESCO.

Figure 6. Locations of wetlands of international importance, as recognized through the Ramsar Convention agreement. Tropical ecoregions, which provide important habitat for a rich diversity of amphibian fauna, are not well represented in the distribution of Ramsar wetlands.

Figure 7. Composited rates of population-, agricultural-, and forest-related change. The resulting patterns show the relative magnitude of the rates at which the landscape is being altered in ways that are likely detrimental to amphibian habitat. The specific maps used to derive the composite map included those showing changes in: human population from 1995–2000, cropland area, fertilizer application rates, irrigated acreage, closed canopy forest, and loss of tree species. Excluded were the maps of agricultural production (changes in production rates do not equate with areal changes in agricultural lands), permanent cropland (it includes only a subset of crop types), natural forests (data were unavailable for many countries), and total forest (it comprises everything from degraded forests, to plantations, to intact, relatively natural forests; from Gallant *et al.* (*submitted*).

Figure 8. Composited rates of landscape change compared with global amphibian distribution patterns (from Gallant *Et al.*, *submitted*).

Chapter 3

Climate Change, Biodiversity Loss, and Amphibian Declines

A. Pounds, A.C.O.Q. Carnaval and S. Corn

3.1 Introduction

The Earth's climate system has witnessed significant changes since the onset of the industrial era. Today's world is warmer and more susceptible to severe climatic events (e.g. heavy precipitation and extreme droughts). Over the 20th century, global mean surface temperature increased by about 0.6°C, which is greater than the change for any other century in the past one thousand years. The daily minimum temperature over land has increased at roughly twice the rate of the daily maximum (Houghton *et al.* 2001). Such warming cannot be explained by natural internal climate variability or external factors such as solar or volcanic forcing. Rather, simulation studies point to anthropogenic activities. By injecting unprecedented quantities of greenhouse gases and sulfate aerosols into the atmosphere, humanity is significantly altering climate on a global scale (Stott *et al.* 2000; Houghton *et al.* 2001; Santer *et al.* 2003; Stott 2003; Barnett *et al.* 2005).

MacCracken *et al.* (2001) and Hulme and Viner (1998) describe potential outcomes for the United States and the tropics, respectively. Global mean temperatures are expected to rise 1.2–3.5° C, but the increase would be higher at mid to high latitudes and greater over continents than over oceans. Warming over the U.S. would be between 2.8° and 5°C, largely due to higher winter and nighttime temperatures. Global precipitation is expected to increase, but predicting local patterns is difficult. Decreased snowfall is expected, reducing the area of snow cover during winter. Higher summer temperatures will increase evaporation, reducing soil moisture, and storms and extreme precipitation events are apt to become more frequent. In the tropics, the predictions are qualitatively similar: increased temperature, increased length of the dry season, decreased soil moisture, and greater inter-annual variation in rainfall. Also, recent trends for tropical highlands suggest reductions in cloud immersion and orographic precipitation as well as increasing cloud-cover frequency (Pounds *et al.* 1999).

These changes come at a cost to natural systems. As we accumulate information from multiple biomes, it becomes increasingly evident that human-driven climatic changes are affecting living systems worldwide. Meta-analyses integrating data from a wide range of taxonomic groups and geographical areas show that global warming is accompanied by shifts in the spatial distribution and range boundaries of several species, as well as shifts in species phenology, with obvious implications for survivorship and reproductive success (Parmesan and Yohe 2003; Root *et al.* 2003; Lovejoy and Hannah 2005; Root *et al.* 2005).

Mounting evidence also indicates that climate changes impact natural communities by affecting how species interact with each other. A particularly pertinent and complex issue with regards to this subject is that of climate-mediated changes in the dynamics or in the strength of interactions between pathogens and their hosts. Pathogens are known to respond to temperature conditions, rainfall patterns, and humidity levels. Climate changes can thus increase rates of pathogen recruitment and disease transmission, and intensify host susceptibility, leading to synergisms that impact biodiversity (Epstein 2001; Harvell *et al.* 2002; Rodó *et al.* 2002). Because of its wide-ranging biological impacts, climate change is expected to accelerate extinction rates for populations and species (Harvell *et al.* 2002; Thomas *et al.* 2004).

Given their complex life cycles and other traits, amphibians are recognized as indicators of ecosystem health. Nearly 33% of the amphibian species of the world are categorized as vulnerable, endangered, or critically endangered as per The World Conservation Union (IUCN). Approximately 43% are experiencing population decreases. Habitat loss and over-utilization explain many, but not all of these declines. Amphibians are suffering widespread extinctions even in seemingly undisturbed environments (Stuart *et al.* 2004).

Evidence indicates that recent climate change is causing some species to breed earlier (Beebee 1995; Gibbs and Breisch 2001; Corn 2003; Tryjanowski *et al.* 2003). Findings also suggest that recent warming trends and superimposed warm episodes of El Niño have caused declines of anurans in Central America (Pounds and Crump 1994; Pounds *et al.* 1999). The mechanisms are not well understood (Corn 2005), yet the climate-linked epidemic hypothesis (Crump and Pounds 1994; Pounds *et al.* 1999; Pounds 2000; Pounds 2001; Pounds and Puschendorf 2004) is an important idea to be tested. Although studies have generally failed to find a link between climate and amphibian declines (Laurence 1996; Alexander and Eischeid 2001), data have not permitted a geographically broad test of such indirect effects of climate change.

In any case, climate change will probably increase pressure on many amphibian species, for a variety of reasons. Reduced soil moisture could reduce prey abundance and eliminate habitat. Reduced snowfall and increased summer evaporation could have dramatic effects on the duration or occurrence of seasonal wetlands, which are primary habitat for many species of amphibians. Because of the rapid rate of warming, many species will be unable to adjust. Historically, organisms have responded to climate change by shifting their distributions. In today's world, physical changes wrought by humans—agricultural development, urbanization, deforestation, etc.—constrain such responses and greatly reduce the pool of replacement populations.

Extinctions in apparently unaltered habitats in different parts of the world render the study of climate change imperative. Pathogens have been frequently implicated in these cases, especially the chytrid fungus, *Batrachochytrium dendrobatidis* (Berger *et al.* 1998; Lips 1999; Kiesecker *et al.* 2001; Blaustein and Kiesecker 2002; Carey and Alexander 2003; Daszak *et al.* 2003; Ron *et al.* 2003; Berger *et al.* 2004; Burrowes *et al.* 2004; Piotrowski *et al.* 2004; Retallick *et al.* 2004; La Marca *et al.* 2005; Ron 2005). However, the relationship between amphibian pathogens and environmental change has not been well studied.

Understanding the synergistic interactions between host-pathogen dynamics and climate change is essential if we are to address the issue of global amphibian declines. For instance, mounting field evidence shows that resistance to the chytrid fungus varies on a case by case basis, and that species which had formerly declined may currently coexist with the disease (Retallick *et al.* 2004), suggesting that other factors might interact with chytridiomycosis. The climate linked epidemic hypothesis predicts that many declines will occur in unusually warm years but is not predicated on a particular disease or mechanism. Nevertheless, chytrid-amphibian interactions provide an appropriate model for examining such relationships (Ron *et al.* 2003; Burrowes *et al.* 2004). Evidence shows that outbreaks of various diseases of humans and wildlife are related to climate variability on the timescale of El Niño, yet examining the relationship to global warming has been difficult because of data limitations (Epstein 2001; Harvell *et al.* 2002; Rodó *Et al.* 2002). A recent study provides strong support for such a relationship in the case of disease-related amphibian declines (Pounds *et al.* 2006).

3.2 Broad Goals and Implications

The findings discussed above highlight the importance of emphasizing research and conservation action related to climate change. Climate change has the potential to wreck havoc all other amphibian conservation efforts. Moreover, the United Nationas Framework Convention on Climate Change (UNFCCC) states that "stabilization of greenhouse gases should occur in a timeframe that allows ecosystems to adapt naturally". If climate change is leading to amphibian declines, this isn't working. Amphibians are warning us about the vulnerability of biodiversity to climate change, and declines are a flashing red light that indicates the need to change public policy. Widespread amphibian extinctions underscore that profound losses of biodiversity are inevitable, unless humanity comes to terms with global warming.

3.3 Key Research Actions: Priority Science Gaps and Future Focus

To address the impacts of climate change on amphibian communities, research is needed to:

1) Understand how climate change affects ecosystems and thus amphibians. Focus should be on changes in disease dynamics and the underlying mechanisms.
2) In light of these mechanisms, identify key elements of climate and quantify the relevant changes.
3) Develop a predictive model of amphibian decline patterns based on these mechanisms and observed trends.
4) Investigate why climate change impacts are worse today than in the past (e.g., landscape alterations, etc.).
5) Examine the context of declines to understand broader implications for biodiversity loss (what are the parallels in other groups).

3.4 Key Conservation Actions

Going beyond research, conservation actions in relation to climate change and amphibian declines need to:

1) Increase public awareness about effects of climate change: create educational/outreach/research centers, web sites, positions in existing institutions.
2) Promote changes in energy policy. Amphibian declines are critical in defining "dangerous human interference" in the climate system.
3) Support initiatives that increase community resilience and reduce sensitivity to climate change (habitat restoration, corridors, etc.).
4) Explore the possibility of manipulating local and micro-scale climate in light of mechanisms identified.

3.5 Logistics

The proposed research component will rely largely on existing field and laboratory infrastructure. Financial support is necessary to fund research projects. A dedicated outreach/education/research center (with ability to hold exhibits, host lectures, enable field and laboratory research, and accommodate visiting scientists) would increase public awareness and allow for interdisciplinary research about climate change and its effects on biodiversity. We can reach out to a large number of people by posting accurate, pertinent information about climate change and its biological effects on a dedicated web page, which can be linked to existing web sites. Outreach and policy change components should be addressed by hiring a dedicated professional (to be affiliated with an existing institution).

3.6 Budget

Table 3.1 Five-year budget for implementing the actions outlined.

Component	Justification	US $
Research: Funding of 20 Ph.D. dissertation grants to address priority science gaps (see Research Goals for a list of end products).	The cost of a research grant is estimated to be US $ 58,550 per year (including stipend, tuition, and funding for research project) x 5 years (average length of Ph.D. study) x 20 dissertations.	5,885,000
Salary for a professional (affiliated with an existing research or policy-related institution) to work for outreach and policy change in relation to climate change (includes working on web site maintenance)	Based on annual salary and travel costs = US $ 75,000 per year.	375,000
Implementation of an outreach/education/ research center dedicated to climate change and its biological effects	Estimate based on an estimated cost of a maximum of US $ 200,000 per year for salaries (up to 10 staff members) x 5 years, + US $ 10,000 costs associated with building infra-structure. After the initial 5-year period, the center should be financially supported through admission fees and donations.	1,100,000
Total for 5 years		7,360,000

Chapter 4

Infectious Diseases

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4.1 Emerging Diseases, Human Health and Conservation

Over the last three decades, there have been an alarming number of high profile outbreaks of new viruses and other pathogens of humans, many of them emerging from wildlife, globally. Diseases such as SARS, avian influenza, HIV/AIDS and others highlight emerging diseases as possibly *the key threat* to the future health of people around the globe. The underlying causes of zoonotic disease emergence usually involve broad environmental changes (e.g., encroachment into wildlife habitat, land use changes), changes to human behavior (e.g., wildlife trade, medical technology) or changes in human demography (e.g., urbanization). These key threats to human survival are therefore clearly linked to broad threats to biodiversity.

The overlap is even more striking when we consider the series of emerging diseases recently reported in wildlife populations. Diseases of species as diverse as corals, African wild dogs, Hawaiian birds, and seals have resulted in mass mortalities, population declines, and even extinctions. Just like their human counterparts, these diseases are linked to broad-scale anthropogenic environmental drivers such as introduction through wildlife trade, forest fragmentation, road-building, climate change and other well-understood environmental threats.

In this chapter, we review the evidence that amphibian populations are also threatened by disease emergence and spread. We propose control measures, research and conservation strategies to address this challenge in a bold, visionary plan to help block disease-related extinctions and understand and prevent future disease emergence.

4.2 Chytridiomycosis

4.2.1 Origin, emergence & distribution

In recent decades at least 43% of amphibian species have declined, 32.5% have become globally threatened, 34 have become extinct, and an additional 88 have possibly become extinct. Rapidly declining species are commonly found in upland Neotropical or Paleotropical riparian habitats, often in protected areas. These declines have been characterized as "enigmatic" due to the lack of obvious cause (e.g., deforestation, introduced predators).

In 1997, an international team comprised of scientists from the USA, UK, and Australia met to compare similar pathological findings in dead and dying amphibians collected in Panama and Australia at sites where enigmatic declines were particularly severe. The result was the description of a new fungal disease of amphibians—chytridiomycosis—associated with simultaneous declines on these two continents (Berger *et al.* 1998). The causative agent, a fungus, was also isolated from a captive population of *Dendrobates azureus* at the United States National Zoological Park and was formally described as a new genus and species *Batrachochytrium dendrobatidis* (Longcore *et al.* 1999). This fungus belongs to a phylum of non-hyphal 'zoosporic' fungi (the Chytridiomycota), most members of which are saprobic detritivores or pathogens of insects; indeed, *B. dendrobatidis* is the first and only known chytrid species pathogenic to vertebrates.

A great deal of work has been conducted on the ecology, biology and impact of this pathogen over the past 9 years. It has been named as an emerging infectious disease, due to its recent spread in new populations (Daszak *et al.* 1999; Daszak *et al.* 2003). Exceptionally low genetic diversity among isolates from Australia, Panama, Ecuador and North America strongly suggests that it has recently emerged as a 'pandemic' disease (Morehouse *et al.* 2003). The wide host range, lack of immune response in affected animals and pattern of die-offs suggest that it has recently spread into the amphibian populations it has most severely affected in Central America and Australia (Daszak *et al.* 1999). The recent emergence of this disease has been linked to globalized trade in amphibians for food, pets and other purposes (Mutschmann *et al.* 2000; Mazzoni *et al.* 2003) and its global distribution may have its origins in the pan-global dissemination of *Xenopus* frogs for pregnancy testing during the 1950s onwards (Weldon *et al.* 2004). It continues to cause severe declines in many parts of North, Central and South America, Europe, and Australia and its distribution appears to be widespread reviewed in Daszak *et al.* (2003). New molecular diagnostic tests have been developed, validated and are now available (Annis *et al.* 2004; Boyle *et al.* 2004), and biochemical studies of amphibian defenses against the pathogen have begun (Rollins-Smith *et al.* 2002).

Despite these advances, there is a great deal that remains unknown about this emerging disease: How does it cause death? Why is it capable of causing the extinction of some populations, while others persist? What is the true underlying cause of its emergence? While work on these issues continues, amphibian populations in some regions have reached a crisis point largely as a result of this disease. Chytridiomycosis is associated with 43 declining amphibian species in seven Latin American countries, and at least 93 species world wide (Berger *et al.* 1998; Lips 1999; Ron *et al.* 2003). The causative agent has been detected in 14 species of *Atelopus* (nine of which have disappeared) and it has been found in at least 38 species of Neotropical frogs (La Marca *et al.* 2005; Lips *et al.* 2005), 49 Australian species (Speare and Berger 2005), 15 species in North America (Speare 2005), 3 species in Africa (Speare 2005), and 20 species in Europe (Garner *et al.* 2005). Given patterns predicted using bioclimatic modeling (Ron 2005), *B. dendrobatidis* is likely to continue to threaten amphibians in many areas of the Old and New World Tropics, including many species in unexplored biodiversity hotspots. Finally, recent data from Central America provide the clearest evidence yet that this disease is the cause of a wave of population declines and probable species extinctions currently sweeping through the region (Lips *et al.* 2006).

4.2.2 Biology and ecology of chytridiomycosis

The pathogen responsible for chytridiomycosis is a zoosporic fungus with 3 life stages: an aquatic, motile infectious stage (zoospore); a parasitic stage found in the skin of adult amphibians (thallus), and the body that discharges new zoospores into the environment (the zoosporangium). The fungus has a high affinity for keratinized tissue in amphibians and causes thickening (hyperplasia and hyperkeratosis) of the *stratum corneum* in metamorphosed individuals and adults. It is also able to infect the keratinaceous mouthparts of larvae, which have no keratin in their skin prior to metamorphosis. It appears not to be pathogenic in larvae, although it can affect their growth rates and other aspects of development (Parris and Baud 2004). In the laboratory, it can be cultured without keratin, using agar-based media or nutrient broth (Longcore *et al.* 1999). It can also survive prolonged periods (up to 8 weeks) as a saprobe living in sterile pond water (Johnson and Speare 2003). Experiments in culture suggest it grows best at relatively cool temperatures (17–25C) with a 4–5 day generation time (Piotrowski *et al.* 2004). It seems to require water, or at least moist conditions, for transmission and development, and dies if desiccated.

These biological traits may help explain its ecological impact on amphibians. Its preference for cool temperatures suggests that it is likely to have a higher impact on amphibians in upland regions of the tropics (Stuart *et al.* 2004). Its ability to grow saprobically without keratin may partly explain its ability to persist at low host densities, such as when a population is almost extinct (Daszak *et al.* 2003). Recent data suggest it can persist in the wild without amphibian hosts for up to six weeks in aquatic mesocosms (M. Parris, unpublished data), and at least three days in tropical moist cloud forest (F. Brem, unpublished data). It has also been found infecting freshwater shrimp at high intensities (Rowley and Alford 2006), suggesting that it may be able to persist indefinitely in alternative host taxa. Other factors that may promote persistence include the presence of less susceptible reservoir hosts e.g., bullfrogs (Daszak *et al.* 2004) and its presence in the mouthparts of larvae, which do not die from infection.

Field studies (Lips *et al.* 2006) indicate that the incidence of chytridiomycosis rapidly expanded within an entire assemblage of montane Central American frogs, causing severe population declines. In such outbreaks, chytridiomycosis appears to emerge at a site and spread by a combination of frog-to-frog and environment-to-frog transmission, as shown in the lab and in field mesocosms. As prevalence increases within the amphibian community, diseased frogs shed zoospores into the environment or directly pass them to other amphibians by contact. We hypothesize that persistence of zoospores in the environment and the long period of infectivity of many amphibians promote saturation of the environment with zoospores. This would produce the pattern we observed in which prevalence quickly changed from very low to very high, followed by widespread mortality. It also suggests a mechanism for the most significant impact of chytridiomycosis—the complete removal of frog populations from a region. Persistence of zoospores outside individual frog hosts, or through active infections in alternative host taxa, would allow infection of amphibians even at extremely low host densities. Furthermore, after chytridiomycosis has removed all of the adult population from a site, persistence as a saprobe or in non-amphibian hosts means that subsequent recolonization attempts may result in spread of the infection to the colonizing population. This may result in longterm extirpation of frog populations from a site, a pattern repeatedly observed in Australia and Latin America (McDonald and Alford 1999; Lips *et al.* 2003; Lips *et al.* 2005).

4.2.3 Detection

Clinical signs of infection in wild animals are either not present, or not obvious until close to death. As the infection progresses, animals may become lethargic, may exhibit increased sloughing of the skin, especially of the feet and ventrum in adults, or may sit in a characteristic posture in which the hind legs and drink patch are elevated off the ground to minimize contact with substrate. Many older tadpoles of some species may show malformations of the keratinized mouthparts, but this is not consistent across species, stages, or habitats and the presence of infection needs to be verified with histology, PCR, or microscopy.

Because of the lack of grossly visible clinical signs, the pathogen needs to be detected by either microscopy (e.g., standard histopathology of the feet or groin skin) or PCR.

4.2.4 Effects on amphibian individuals and populations

Surprisingly little is known about how the disease causes death. The thickening of the keratinized layer of the skin (epidermal hyperkeratosis) may hinder osmoregulation or respiration through the skin to some degree. Recent unpublished work suggests that infections lead to a breakdown of ion uptake, affecting osmoregulation and eventually killing the host. Many factors are thought to affect the outcome of infections, including host behavior (R. Alford, unpublished data); environmental factors (temperature and others); host physiology, life history, geographic and elevational ranges, physiology, and innate immune response via skin peptides.

At population scales, the impact probably depends primarily on the species of amphibian infected, contact rates between individuals, external environment, host behavior and pre-exposure. Probably the most significant factors in determining whether a species will decline or persist in the face of chytridiomycosis are host ecology, specifically niche specialization, fecundity, habitat (stream vs. non-stream) (Williams and Hero 1998). Whenever mass die-offs have been observed in Latin America, population declines were rapid (4–6 months maximum) and over 50% of species were extirpated, remaining species persisted at ~20% of normal abundance, and any recovery that might occur can take from 4 to more than 15 yrs post-decline. Documented declines in Australia have occurred even more rapidly, with populations falling from normal levels to zero over 6–8 week periods (McDonald and Alford 1999). Responses of amphibian populations fall into one of three categories (Lips *et al.* 2003): complete removal of population (montane endemics, specialist, rare, riparian species); declines but persistence (intermediate traits); no decline (widespread, lowland, common, generalist species). A few species may carry sub-clinical infections and act as vectors, including the bullfrog (*Rana catesbeiana*) and the marine toad (*Bufo marinus*), *Xemopus* spp., and tiger salamanders (*Ambystoma tigrinum*), all of which have been introduced to new areas (Collins *et al.* 1988; Mazzoni *et al.* 2003; Hanselmann *et al.* 2004; Weldon *et al.* 2004). There is a recent report of amphibian populations that have persisted following die-offs, while still harboring chytridiomycosis (Retallick *et al.* 2004). This suggests a possibility of hosts developing resistance, or at least tolerance of the pathogen.

4.2.5 Preventative strategies for emerging diseases of wildlife

Dealing with a wildlife emerging disease is a problematic area, both ethically and logistically. Indeed, there have been few direct intervention efforts to prevent the spread of disease in wild populations of animals. A relatively successful example is the oral vaccine baiting for rabies that has been conducted to curtail the spread of raccoon rabies in the northeastern USA (Russell *et al.* 2005). While successful for some time, the barriers have recently broken down and the cost is high. Another example, that of vaccination of African wild dogs in the Serengeti National Park for rabies, proved ethically challenging, although the current strategy of vaccination of domestic dogs surrounding the park has reduced rabies prevalence in wild dogs (Woodroffe 2001).

There are no currently available vaccines for chytridiomycosis and development of a vaccine has not yet been planned, and would likely take many years. The treatment of amphibians in the wild with anti-fungal agents would also be problematic: release of anti-fungal agents in a way that would target the frog population significantly enough to deal with infections would likely cause catastrophic side-effects to the fungal component of the ecosystem. Therefore, simple population management strategies are the only viable option. These may include capture of wild individuals, treatment with drugs that kill *Batrachochytrium dendrobatidis* (Parker *et al.* 2002) or heat, which can kill the fungus (Woodhams *et al.* 2003), then breeding in captivity ready for release into an area deemed free of disease. Collecting animals for survival assurance colonies may be timed to move ahead of any direction of epidemic spread. Disinfection of footwear with 10 percent chlorine bleach solution to prevent the spread of diseases by tourists and other people moving into sites with 'at risk' populations has been proposed (www.nwhc.usgs.gov/research/amph_dc/sop_mailing.html). The recent discovery that bacteria that inhabit the skins of frogs and salamanders can inhibit the growth of *B. dendrobatidis* in vitro (Harris *et al.* 2006) suggests that biological control of the pathogen may eventually be possible; however this will require a substantial body of further research.

One of the most important strategies to help mitigate the impact of chytridiomycosis is to develop the infrastructure for surveillance and population management at the sites that are likely to be affected by this disease in the future.

4.3 Other Diseases

4.3.1 Ranavirus infections

Ranaviruses are a frequent and increasingly-reported cause of amphibian mass mortality events. Such ranavirus epidemics are usually characterized by explosive die-offs, often with extremely high mortality rates. Ranaviruses are, therefore, candidate agents of amphibian population declines.

Ranaviruses are viruses in the genus Ranavirus, family Iridoviridae. They are large (120 to 300 nm diameter), icosahedral, linear double stranded DNA viruses. The type virus of the Ranavirus genus, Frog Virus 3 (FV3), was isolated in 1965 from the leopard frog (*Rana pipiens*) in North America and has since been shown experimentally to kill frog embryos and tadpoles. Tadpole Edema Virus (TEV), originally isolated from grossly oedematous wild North American bullfrog (*Rana catesbeiana*) tadpoles, has been shown by transmission experiments to be the aetiological agent of this disease following transmission experiments. Also, focal hemorrhages in the viscera and muscle were seen in animals (*Scaphiopus* sp. and *Bufo* spp.) infected experimentally with this virus.

More recently, iridoviruses have been isolated with increasing frequency from amphibians that died during mass mortality events in Australia, North America and in Britain. Additionally, ranaviruses have been isolated from amphibians in South America, but no disease or outbreaks of mortality have so far been associated with ranavirus infection on this continent.

4.3.2 Ranaviruses in North America

After the almost contemporaneous discovery of FV3 and TEV (now considered to be a strain of FV3) no further cases of ranavirus disease were detected in amphibians in North America until the 1990s. Since then, however, numerous ranavirus epidemics have been recorded in amphibians on this continent, with disease outbreaks having so far occurred in at least 22 U.S. states and three Canadian provinces. The infection frequently causes mortality rates in excess of 90% in a pond. In at least six states (Arizona, Maine, Minnesota, North Carolina, North Dakota, and Wyoming), four or more ranavirus-associated mortality events have occurred since 1995, with several sites in these states experiencing two to eight consecutive years of mortality events at essentially the same month each year. In North America, the most commonly affected genera are *Ambystoma, Pseudacris,* and *Rana*, but other genera, such as *Bufo, Hyla,* and *Notophthalmus* are also affected.

4.3.3 Ranaviruses in Australia

In 1992, the isolation of an iridovirus (Bohle iridovirus, BIV) from the ornate burrowing frog (*Lymnodynastes ornatus*) during an investigation into deaths of recently metamorphosed frogs in Australia was reported. To date, BIV has been experimentally transmitted to a wide range of amphibian species and to barramundi fish. Although ranavirus has been isolated only from a single outbreak of disease in wild amphibians in Australia, ranavirus antibodies have been reported from the introduced cane toad (*Bufo marinus*) throughout its Australian range. This suggests that ranavirus is more widespread in Australia than evidence based on disease occurrence indicates. There is, however, no evidence of amphibian population declines in Australia caused by ranavirus infection.

4.3.4 Ranaviruses in Europe

On-going recurrent annual epidemics of mass mortality of anurans (primarily of *Rana temporaria*, but also affecting *Bufo bufo*) due to ranavirus infection have been reported from the British mainland since the early 1990s, with most reports centered on the south-east of England. Although there is some evidence of these outbreaks causing local declines and even population extirpations at some breeding ponds, the effect on amphibian populations overall is unknown.

An iridovirus (since shown to be a ranavirus) was isolated from edible frogs (*Rana esculenta*) with systemic haemorrhages and skin necrosis, but transmission experiments using cultured virus failed to reproduce disease. No further reports of ranavirus-associated mortality are known from Europe.

4.3.5 Impact of Ranaviruses on amphibian conservation

In the USA, ranavirus epidemics usually affect late-stage frog or salamander larvae or recently metamorphosed animals, while in Europe most reports are of adult anuran mortality. A major pattern of ranaviral mortality events is the abundance and widespread distribution of the affected species. With only one possible exception, none of the affected species are considered to be in decline and none are listed as threatened or endangered species. This pattern suggests that ranaviral mortality events might be a disease of population abundance or crowding. The one species to experience a ranaviral mortality event that also is suspected to be a species in decline is the mountain yellow-legged frog (*Rana muscosa*). Because thousands of sick and dead tadpoles were found during the die-off, it is likely that a high population density (crowding) existed at the site. In conclusion, there is no apparent association between the occurrence of ranavirus-associated mortality and amphibian declines. However, the recent publication of the full genome sequence of one amphibian ranavirus (Jancovich *et al.* 2003) provides for the capacity to probe this as a model for looking at other, more important amphibian diseases from a conservation perspective (Collins *et al.* 2004). Studies of ranaviruses may also act as a 'control' for studies of emerging epidemic diseases, because some ranaviruses clearly exist in an endemic disease state in wild population.

4.3.6 Other diseases

A wide range of pathogens that affect amphibians has been described. In this report, we have focused only on those where a conservation impact has been clearly shown. However, some pathogens that are known to science (e.g., fungal *Saprolegnia* spp.), and others that have not yet been described, may be implicated in declines. Clearly, there is a pressing need to continue to collect baseline data on known and unknown pathogens of amphibians, globally.

4.4 Complexity and Synergism in the Disease Threat

There are increasingly clear links between the emerging disease chytridiomycosis and a series of other threats to amphibians. Habitat loss is a major threat to amphibians globally, and where habitats are fragmented, and populations diminished, they become more susceptible to disease outbreaks. Toxins in the environment may act synergistically with pathogens. Farming of bullfrogs and their international movement in the wildlife trade may be helping to spread chytridiomycosis internationally. Finally, climate change appears to be playing a role in extinctions in one of the regions that chytridiomycosis is currently implicated in causing declines and extinctions—Central America. There, climate change may be one of the underlying causes of disease emergence, with habitat loss, trade and other factors adding to this complexity. Disease issues are also clearly linked to the need for captive survival assurance programs to clear animals of infection, produce disease-free progeny and ultimately reintroduce them into habitats that are free of virulent pathogens. A major priority of our global plan, below, is therefore to link synergistically with the other priorities of ACAP.

4.5 A Global Plan to Combat Disease Threats to Amphibians

4.5.1 Regional centers for chytridiomycosis control

For cultural and logistical reasons, we propose regional centers for the control of chytridiomycosis at areas most likely to be hit hard by the disease. These will be globally representative, including sites in Latin America, Australia, and Europe, where highland amphibian species are under severe threat from disease and Africa and Asia where we do not know enough about the threat of disease. These centers will have basic laboratory facilities for culture, diagnosis, treatment and housing of breeding stock of local amphibians. They will be focal points for scientists from other regional centers, and from other countries to visit and conduct fieldwork, or assist in developing preventative and control strategies.

Each regional center will act as a diagnostic lab with state-of-the-art molecular equipment and trained staff (molecular and database/GIS technicians) to process samples and track infections in liaison with other local scientists, conservation biologists and ecologists and other regional centers. These facilities will have the capacity to test 1000 samples for *B. dendrobatidis* per month. They will establish a no-fee service lab to conduct PCR-based detection analyses for samples from around the region they represent. Funding made available for these free tests will be recommended by a panel of experts based in the region, using priority decision trees based on modeling of conservation risk, patterns of disease outbreaks and other priorities. These regional panels will accept proposals to request a number of free tests and decide which project will be allocated free tests, and the number of those. We anticipate that data from all samples submitted would be added to a developing central database that would represent the current status of the collective sampling efforts of biologists and our knowledge of the current presence of *B. dendrobatidis* across Latin America. Individuals submitting samples would have the choice after their samples have been analyzed to have them destroyed, returned to them, or to be added to a voucher collection that would be available for genetic analyses of *B. dendrobatidis* available to all qualified investigators. Intellectual property rights issues would be dealt with on a local, regional basis, by the researcher submitting samples working with the regional laboratory. The capacity for these facilities exists in many forms around the world, and rather than creating new labs, it may be more efficient to fund the expansion of existing labs.

These regional centers will be the home base for rapid-response teams comprising teams of leading, local microbiologists, veterinarians, wildlife biologists and ecologists that will travel to sites where outbreaks are clearly occurring or are suspected. These teams will conduct outbreak investigation programs in the same way that the US-based Centers for Disease Control and Prevention and the Australia-based CSIRO Australian Animal Health Laboratory do so for human emerging diseases. They will also conduct intensive biotic surveys of amphibians along elevational and latitudinal transects to describe the geographic distribution of the problem, identify potential causes, help set priorities for conservation efforts, assist local scientists in surveying for amphibians, pathogens, and other signs of amphibian declines. These rapid response teams are envisaged to be composed of scientists already salaried, but supported by travel funds, funds for logistical support and for consumable and testing costs.

4.5.2 How do amphibian emerging diseases spread, cause die-offs and cause extinctions?

When medical research deals with emerging diseases of people, the first step is invariably to understand why the disease is spreading and why it is causing deaths within the population. We propose an ambitious research agenda directed to understanding why some populations and species of amphibians become extinct in some regions, whereas others do not, even when faced with the same emerging disease. This ecological research agenda will include studying persistence of the pathogen, reservoir hosts, mechanisms of spread, interactions with climate change and models of disease dynamics. Crucially, these studies will be targeted to 1) sites where amphibians are undergoing enigmatic declines due to chytridiomycosis, linked with studies of climate change, habitat loss, etc., and 2) sites where *B. dendrobatidis* is present, yet populations of amphibians persist without declines.

More research into the ecology of *B. dendrobatidis* is needed, including such basic and critical aspects of its natural history as how and where it survives and how long it can persist in the environment. Disease risk maps, such as one already produced for the New World (Ron 2005), are commonly used in human emerging diseases and may here serve as a framework for setting research and management priorities to control the spread of this disease and to safeguard threatened amphibians. One of the highest priorities is to determine the means by which *B. dendrobatidis* moves among sites, species, and individuals over local, regional and international scales. Monitoring of the trade in amphibians, testing animals throughout that trade and dealing with the policy implications of trying to block disease in those trades is a key priority. Despite our knowledge of many aspects of amphibian biology (Duellman and Trueb 1986), basic ecological and natural history data are lacking for most tropical species. Studies of chytridiomycosis ecology will, therefore, integrate with those on amphibian diversity and ecology. There is clear linkage between disease and climate change and other factors. Studies of the ecology of chytridiomycosis and other diseases should include broad surveys of its altitudinal and latitudinal distribution and impact, modeling of amphibian population responses to climate change and how this alters disease dynamics, study of the relationship between its spread and trade in amphibians and other key issues. Disease researchers will provide detailed data for modeling by climate groups and testing hypotheses on spread and impact under different climate change scenarios.

Finally, we need to continue to survey museum collections and conduct molecular phylogenetic studies to find out when and where *Batrachochytrium* first emerged or whether its distribution has always been wide, and to survey where it is now, in areas with either declining or stable populations. Systematists will be encouraged to work with disease researchers to identify declines consistent with disease and to help sample for disease in collections. Part of this research agenda will be to continue to develop cheaper and more efficient testing methods for biological and environmental samples—products that will benefit reintroduction, disease outbreak investigations, as well as survey programs.

4.5.3 Knowing the enemy—tackling the biology of chytridiomycosis

While we have gained a great deal of information on chytridiomycosis over the last few years, there is a pressing, urgent need for research on specific aspects of its biology in the host and in the environment. Understanding how amphibians respond to infection is a critical goal. Do they become immune when first infected, or are they susceptible even after they have cleared an initial infection? Do amphibians respond to infection by changing behavior, e.g., basking, to eliminate infections? Addressing these questions will involve simple captive studies of live amphibians. The ethical challenges of conducting these will be reviewed and dealt with by representatives of the ACAP group as the projects progress (Minteer and Collins 2005). Studies of how temperature affects pathogenesis and virulence and affects saprobic behavior will be conducted in the lab and fed into models of how chytridiomycosis might respond ecologically to climate change. Another key, unresolved issue is how chytridiomycosis causes death. Does it affect the ability of frogs to respire or osmoregulate through their skin, or does the pathogen release toxins that ultimately cause death? Sophisticated approaches to dealing with these issues are already underway and support is urgently required. Little information exists on the physiological tolerances, immune responses and other aspects of tropical amphibian biology. The research proposed here will be targeted to amphibian species from each region, including species in decline, but also "control" species that are tolerant (e.g., the bullfrog *Rana catesbeiana*) or able to clear infection (e.g., *Ambystoma tigrinum* and *Pseudacris triseriata*). This work will also focus on representative species globally.

The ultimate goal of this work will be data that can help produce practical conservation outcomes that bolster the ability of amphibians to deal with disease. To this end, seed money will be made available for experimental approaches to develop simple, imaginative solutions to treatment and control that will increase the capacity and reduce costs of keeping captive assurance colonies free of disease. These will include, for example, examining how microbes compete with *B. dendrobatidis* in the environment and on amphibians, and trials of heat-treatment techniques in mesocosm and wild settings.

4.5.4 Strategies to deal with disease in the wild

Dealing with an emerging disease in wild animals will require "outside-the-box" thinking. It is a tough challenge, both logistically and ethically, to prevent the spread of a pathogen in a wild population, especially where there is little infrastructure, as in many of the current decline sites. We propose a seed funding system for imaginative approaches to treating animals in the wild, modifying habitats to curtail disease spread (e.g., treating vehicles and people to reduce risk of pathogen dispersal) and other procedures to prevent extinction by infection. One crucial part of the armory is understanding why some species are tolerant (e.g., bullfrogs), able to clear infection and recover (e.g., salamanders), or completely resistant to infection. Studies of how these species' immune responses, behavior, antimicrobial peptide responses, or genetics differ from susceptible species will be crucial to developing such strategies, and is the first, most critical phase of the effort. Other strategies may involve captive breeding to select for resistance to *B. dendrobatidis* and other diseases, or even biological control or release of genetically modified pathogens or frogs, while assessing the ethical and conservation implications of releasing such animals back into the wild. These ethical issues are at the heart of this approach and will be at the forefront of this part of the program (Minteer and Collins 2005). Finally, close linkage and synergism with the captive assurance colony and reintroduction component of ACAP is clearly fundamental to dealing with chytridiomycosis in the wild. The captive assurance colony component of ACAP is one of the most immediately critical issues for some regions where chytridiomycosis threatens amphibians (e.g., Latin America, Australia).

4.6 Budget

Outlined below is an ambitious budget that covers an imaginative, visionary agenda to address one of the most critically challenging threats to global amphibian declines and extinctions. Some of these issues are direct responses to threats in the wild, and others involve high priority research to understand the biology and ecology of the emerging pathogen, *B. dendrobatidis.* Proposed actions include imaginative, controversial and ethically challenging approaches to treatment of amphibians in the wild. It is important to note that these are equally critical to amphibian conservation. If the research proposed is not done, we will be in the same position as we are now, with disease-contaminated habitats where amphibians are being reintroduced.

4.6.1 Regional centers for disease diagnostic

Table 4.1. Cost of establishing and maintaining regional disease diagnostic centers with a capacity of 1000 samples per month each for five-years.

Region	US $
North America	1,750,000
Europe	1,750,000
Australia	1,750,000
Latin America	885,000
Asia	885,000
Africa	885,000

- Rapid response teams (5 x $50,000)—one for each regional center: $1,250,000

4.6.2 Monitoring to understand origin of emerging diseases

- Museum surveys $ 1,000,000
- Monitoring of trade (in conjunction with over-harvesting group) $ 1,000,000
- Development of more rapid and cheaper field tests $ 1,000,000

4.6.3 Disease ecology

- Pathology and epidemiology of B. dendrobatidis. Studies on $ 5,000,000 transmission, persistence, reservoir species, and variation among sites and host-taxa? Epidemiological and environmental modeling.

4.6.4 Disease biology to develop control strategies

- Behavioral response to infection $ 500,000
- Seed money for microbial interactions with B.d. $ 400,000
- Pathogenesis and cause of death $ 750,000
- Simple experiments to assess immune response in amphibians from each region $ 750,000
- Antimicrobial peptide work $ 400,000

4.6.5 Developing strategy for population scale control

- Selection for resistance in frogs from each region $ 1,000,000
- Understanding why some species are tolerant (e.g., bullfrog),some recover (e.g., salamanders), and some are resistant—AMPs, behavior, host genetics, microbial issues) $ 4,000,000

  Seed funding for alternative solutions (e.g., biological control) $ 500,000

4.6.6 Total Five-year Budget $ 25,455,000

Chapter 5

Over-harvesting

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5.1 Introduction

The global human population continues to grow, resulting in concomitant increases in the use of natural resources. Government bodies, especially those in developing countries, have limited resources for implementing effective conservation measures to mitigate the impact of these increasing pressures on both habitats and species (Rowcliffe *et al.,* 2004; Carpenter, 2006). Therefore, the protected area approach towards conservation is difficult to enforce, especially where governance implementation is weak. This is further complicated by the fact that many endangered species are often recorded outside protected areas (Bruner *et al*., 2001; Chape *et al.* 2005). A suggested solution to this problem has been to set up wildlife use programs that directly benefit the local people whose actions are often negatively impacting biodiversity. By giving these people an economic stake in the harvesting of a species it is anticipated that this should provide a sufficient incentive for conserving both the species and its habitat.

For any such conservation project to be truly beneficial it must be sustainable in the long-term, but natural populations are often harvested without adequate consideration of either the implications for the dynamics of the exploited population, or the potential impacts of alternative harvesting strategies (Gezt & Haight, 1989). Population models that incorporate harvesting are often simplistic, ignoring important ecological aspects, such as stochasticity, age structure and spatial patterns, as well as economic aspects, such as labour, capital and price dynamics (Sinclair *et al*.. 2006). Such factors have been addressed individually in the literature (Cohen, 1987; Andersen & Sutinen, 1984; Clarke & Reed, 1990; Watkinson & Sutherland, 1995) but multi-factor approaches are a more recent development (Francis & Shotton, 1997; Carpenter *et al*., 2004, 2005). The consideration of multiple factors can have important implications for the sustainability of harvesting (Getz & Haight, 1989; Milner-Gulland & Mace, 1998; Reynolds *et al*., 2001). Thus, there is much potential for improving our current understanding of harvesting impacts in complex environments. Impacts that are further complicated by the need to link population models to the 'real world', where harvesting strategies are constrained by the social and economic factors that affect the people who both carry out, and benefit from, trade in wildlife products.

The success of any wildlife trade project is further affected by confounding factors, such as supposedly 'community based' projects being driven solely by 'outsiders' and, thus, not by the community themselves. It is also important to remember that projects set up solely by natural scientists risk failure through a lack of rural development understanding or local support. Conversely, any project set up by development workers with little or no knowledge of how to obtain and interpret ecological and/or population data risks ecological unsustainability. It is also essential that every effort is made to transfer knowledge to local people, because if the project is run solely by NGO staff, then the project will collapse after the departure of the NGO. Successful development projects often have to overcome political and technical problems and whilst some might regard community-based conservation as a new paradigm, it certainly cannot be considered a quick fix solution that is applicable to all situations. It is important to note that several studies have already commented that previous community-based conservation projects have been unsuccessful in terms of both conservation and development objectives (Songorwa, 1999; Western, 1982; Milner-Gulland & Mace, 1998), which highlights the difficulty in achieving success.

This chapter provides a global perspective of the effects over-harvesting has on amphibians using case studies from three countries representing three continents; Asia (represented by China), Americas (Mexico) and Africa (Madagascar). Recommended conservation measures and a budget will also be provided as a means to identify and support critical factors for conservation action.

5.1.1. Amphibians as biological resources

Flora and fauna species are renewable resources, with species often categorized into either *r* or *k* strategists. However, when taxa are investigated in detail, greater variability than the earlier dichotomy provides is often observed between species and even populations, such as breeding and mating strategies used by amphibians (Pough *et al.* 1998). It is essential, when considering any harvesting strategy, that information exists for factors such as population size, breeding strategies, fecundity and survival rates (Resit Akakaya *et al*., 1999). Ideally for a coherent management plan, the following scientific information should be known about the species proposed to be harvested:

- species:environment relationships;
- population density;
- population sex ratio;
- fecundity rate;
- life history stages; and,
- survival rates at each life history stage.

The recommended information highlights the need to conduct species-specific monitoring before considering a species for harvesting. However, such information is often overlooked, while the monitoring is often also neglected during and post-harvesting phases. Where monitoring does take place, the data may be collected over too short a time interval or using unsuitable sampling methods.

The long-term consequences of harvesting on wild populations depends upon a wide range of factors, such as the frequency and season of harvest (Freckleton *et al*., 2003), and the life stage or age of individuals collected (Cameron and Benton, 2004). Predicting the consequences of harvesting involves an understanding of the harvesting schedule together with the demographic rates of the species, and how they are affected by population density (Freckleton *et al*., 2003), as it is these factors that determine population growth rates.

5.1.2. Amphibians as economic resources

Amphibians are economic resources and, thus, are renewable if harvested sustainably. There are two main economic categories into which an amphibian species may be assigned (Perman *et al*., 1999): species that provide direct benefits to the stakeholders (direct use), and species where benefits are accrued in a non-direct manner (indirect use). There are some important economic concerns that must be considered prior to establishing a sustainable harvesting project including stakeholder interests, economic structure, trade network structure, resource substitutability, local and international governance and local people's incomes (Perman *et al*., 1999; Sinclair *et al*., 2006; Carpenter *et al*., 2005).

Amphibians have been recorded as resources for food, leather, the pet trade, medicinal products, etc (Pough *Et al.*, 1998) (Table 5.1). Species commonly used for meat, especially frogs' legs, are *Rana catesbeiana*, *R. esculenta*, *R. tigrina, Pyxicephalus adspersus, Limonectes macrodon* and *Fejervaya cancivora*. It has been claimed that in the early 20th century hunters in Florida could earn up to US $500 per year hunting frogs and in 1976 2.5 million kilograms of frogs' legs were imported to the USA mainly from Japan and India (Pough *et al.*, 1998). Since 1987, India has ceased exporting frog legs, however, an estimated 200 million pairs of frogs' legs were imported annually from Asia to the United States (Pough *et al.*, 1998). Similarly, there are records of frogs being used as purses, key cases, and other novelties and curios, while skins are used in the 'leather' and glue trade (Pough *et al.*, 1998). Individuals are collected for the pet trade also, with nearly 41,500 individuals removed in just 1990 from the wilds of Florida (Pough *et al.*, 1998). Certain amphibian species are also used in traditional medicine; in China, for example, 32 species are now recognized to be of medicinal value in traditional Chinese medicine (Ye *et al.*, 1993). The ways in which amphibians are used varies across the regions of the world; for instance, human consumption of amphibians is relatively high in the Indomalayan and Palearctic regions while the use of amphibians as pets is relatively high in the Neotropical region (Table 5.2).

5.1.3. The impact of trade on amphibians

Trade has been identified as a major driver in the global decline of amphibians (Gibbons *et al.*, 2000). However, the actual impact of the wildlife trade on amphibians is unknown as there is usually insufficient data on wild populations and their trends. While conservation benefits are often sought from the sustainable exploitation of natural resources (Norman, 1987; Carpenter *et al.*, 2004, 2005), it is unclear what levels of exploitation are appropriate for amphibian populations in different regions. Whilst some reptiles have the capability to withstand high levels of harvesting because of their rapid growth rates, early maturation and high fecundity (Shine *et al.*, 1999), such factors are often not known for most species of harvested amphibians. Some data do exist, however, and there is evidence that the trade in some species of frogs' legs have resulted in levels of exploitation that are unsustainable, e.g. *Rana* e*scuelenta* from Europe. In order to continue supplying the growing demand for this market, breeding farms have been established that import and breed non-native species, such as the American bullfrog *Rana catesbeiana.* Some of these animals escape or were intentionally introduced into the wild and may impact on native species. Indeed, in the United States, this species has displaced *Rana pipiens* from their native state of Nevada and *Rana*

Table 5.1. Purposes for which amphibians are used according to the Global Amphibian Assessment (GAA); figures indicate number of species.

Purpose	Subsistence	Sub-national/National	Regional/International
Food – human	212	66	20
Food – animal	5	1	0
Medicine - human and veterinary	66	32	11
Poisons	5	0	2
Wearing apparel, accessories	1	0	0
Handicrafts, jewellery, decorations, curios, etc.	2	2	1
Pets/display animals, horticulture	24	88	261
Research	1	18	11
Sport hunting/specimen collecting	2	15	10
Other	5	1	0

Subsistence = Subsistence use/local trade (generally implies direct use by the harvester/family/local community; includes barter for other locally-produced goods, but not sale for profit)
Sub-national/national = Sub-national/national trade (commercial trade, i.e. involving sale/barter for profit, without crossing international borders)
Regional/international trade (commercial trade crossing one or more international borders)

Table 5.2. Bioregional breakdown of the purposes for which amphibians are used with the number of threatened species included in the trade shown in ().

Purpose	Afrotropical	Australasia	Neotropical	Palaearctic	Indomalayan	Nearctic
Food - human	25 (4)	15 (0)	44 (21)	88 (24)	112 (22)	13 (5)
Food - animal	0	1 (0)	2 (1)	3 (0)	1 (0)	0
Medicine - human and veterinary	3 (0)	0	19 (13*)	45 (10)	27 (2)	2 (1)
Poisons	0	0	7 (0)	0	0	0
Wearing apparel, accessories	0	0	1 (0)	0	0	1 (0)
Handicrafts, jewellery, decorations, curios, etc.	0	1 (0)	2 (0)	0	0	1 (0)
Pets/display animals, horticulture	61 (15)	12 (3)	131 (38*)	54 (15)	31 (7)	14 (2)
Research	2 (0)	2 (2*)	11 (1)	8 (1)	7 (1)	4 (1)
Sport hunting/specimen collecting	0	0	1 (1)	9 (9)	1 (1)	8 (4)
Other	0	2 (0)	2 (0)	1 (0)	2 (0)	1 (0)

boylii from California. There is also evidence that introduced species can introduce novel diseases. This problem could be solved by breeding native species for human consumption instead of introducing exotic ones (Casa *et al.*, 2005).

The level of impact that exploitation has on a particular species is influenced by its ability to respond – and possibly adapt - to its removal from the wild. If unregulated, over harvesting can remove too many breeding adults and decimate small or isolated populations and severely reduce the viability of larger populations. However, a recent study showed that *Fejervaya cancivora* may be able to withstand high levels of off-take due to its high reproductive rate, long breeding cycle and ability to withstand harsh conditions in man-made environments (Kusirini & Alford, 2006).

Rising demand together with increased protection for certain sought-after species can lead to an increase in illegal off-takes both locally and internationally. Even if the initial trade targets abundant species at the onset, this may be followed by reduced numbers of the target species and increased numbers of rare species as the trade exhausts the available supply. The impact of the wildlife trade on species also depends upon what proportion of the trade is sourced from wild, captive bred or ranched animals. The wildlife trade is monitored by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Countries trading in wildlife resources provide data both on the source of individuals and on the levels of trade in reports submitted to CITES (Harwood, 1999; Carpenter, 2003).

5.2 Case Studies

5.2.1 China

The use of amphibians in China poses a serious threat that adversely affects eighty-four species from nine families (Table 5.3).

Since China joined CITES in 1981, it has listed the Giant Salamander on Appendix I and several *Tylototriton* spp. and *Hoplobatrachus rugulosa* on Appendix II (UNEP-WCMC 13 December, 2006. *UNEP-WCMC Species Database: CITES-Listed Species).* Still, it is difficult to protect these species because law enforcement is inadequate, illegal collecting remains widespread and there is a great domestic demand for these species. The main uses are for the food, medicinal and pet trade.

From the wide variety of amphibians harvested for food, the most popular species are *Hoplobatrachus rugulosa*, found in lowland wetlands and paddy fields in South and Central China, *Pelophalx nigromaculata* and *P. planci* found in paddy fields and ponds in Central and Northeast China, and almost all of the stream-dwelling *Paa* species from South and Central China. Population sizes of all these once common frogs have declined considerably due to over-exploitation and habitat degradation (Ye *et al.*, 1993), such as the threatened Giant Salamander that can weigh up to 40 kg and is considered a delicacy with a high market value. This species is particularly vulnerable because of its relatively long life cycle preventing it from recovering quickly from harvesting pressures.

Amphibians and their body parts are traditionally used by diverse ethnic groups for medicinal purposes. No large-scale population declines have been reported but local population declines are noted in places where the harvest is unsustainable. However, the *Batrachuperus* salamanders have a rather restricted range and low fecundity; collecting for medicinal use and food has already caused a population decline (Ye *et al.*, 1993). Another species, *Rana chensinensis* is widely distributed from Central to Northeast China, but over-collecting and habitat destruction has caused population crashes across its range (Ye *et al.*, 1993).

Amphibian trade for the pet market is not considered to be high, except for certain *Tylototriton* salamanders that have restricted distribution, low reproductive potential and are subject to other collecting pressure.

The suggested conservation actions for China include:

- Researching the viability of establishing long-term, sustainable harvesting methods;
- Developing Species Action Plans for threatened species, such as the Critically Endangered Giant Salamander;
- Monitoring trade with an emphasis on the domestic trade;
- Establishing a workable certification system for commercial breeding enterprises;
- Strengthening the law enforcement and regulations to prevent over-harvesting and collecting within nature reserves; and,
- Raising public awareness within China about amphibian decline.

5.2.2 Mexico

Mexico has 364 amphibian species, of which 128 are endemic. There are 183 species protected under national law. Mexico joined CITES in 1991 and since then, four species have been listed as Appendix II (*Ambystoma dumerilii, A.lermaense, A. mexicanum and Bufo retiformis*). There are no species listed under Appendix I (UNEP-WCMC. 13 December, 2006. *UNEP-WCMC Species Database: CITES-Listed Species*).

Between 1995 and 2004, there were export permits granted for *Ambystoma dumerilii* (live specimens=8), *A. mexicanum* (live=8, derivatives=11, eggs=200) and *Bufo reformsi* (unspecified=3). Between

Table 5.3. The impact of utilization on amphibians in China.

Family	Number of species adversely impacted by utilization	Number of rapidly declining species* threatened by utilization	Number of category deteriorations for utilized species
Bombinatoridae	2	0	0
Bufonidae	4	0	0
Hylidae	2	0	0
Megophryidae	8	0	0
Ranidae	39	12	21
Rhacophoridae	3	0	0
Cryptobranchidae	1	1	1
Hynobiidae	12	1	2
Salamandridae	13	2	2
Total	84	16	28

* *Rapidly declining* species are those that have deteriorated in Red List Category since 1980. One *category deterioration* is a movement of a species by one Red List Category since 1980. For example, a movement of one species from Least Concern to Near Threatened is one *category deterioration.* A movement of two species from Least Concern to Near Threatened is two *category deteriorations.* A movement of one species from Near Threatened to Critically Endangered (as is the case with *Andrias davidianus*) is three *category deteriorations.*

1995 and 2004, a total of 477 specimens comprising at least eleven different genera were confiscated within Mexico. The most frequently confiscated species included *Gastrophryne elegans*, *Bufo debilis*, *Hyla eximia* and *Ambystoma* spp.

Amphibians have suffered serious population declines in Mexico and over-exploitation is a principal threat. The most popular uses of amphibians in Mexico are for food, traditional medicine, witch craft and art crafts, scientific research and the pet trade. Between 1987 and 1998, a total of 197,086 *Ambystoma dumerilii* individuals were extracted from Lake Paztcuaro in Michoacan. This lead to a prohibition being imposed by the Mexican authorities in 1998; which in turn resulted in fisherman no longer reporting how many individuals were being collected. It was later discovered through interviews with the fishermen that they were still collecting this species, mostly as a food source or for medicinal uses. Between 1977 and 1978, 26% of 1,094 tons of frog meat consumed globally came from Mexico (DIAPROY, 1998).

The pet market for amphibians in Mexico is relatively small and locally based. The most important species in the pet trade are: *Hyla eximia* (± US $2 per 20 frogs per bag), *Pachymedusa dachnicolor* (±$5 per individual), *Bufo marinus* (± US $5 per individual) and *Ambystoma mexicanum* (± US $10 per individual).

Suggestions for conservation action in Mexico include:

- Developing of amphibian identification and management courses for wildlife inspectors including those belonging to the Federal Ministry for Environmental Protection;
- Creating an awareness program that includes informative posters and brochures on amphibians and the threats they face, and discussions with local communities on topics such as the importance of amphibians, threats they face and how to help them;
- Evaluating the abundance of amphibians which are utilized in order to create sustainable management plans, captive breeding centres or community farms;
- Designing an inspection program for the Conservation, Management and Sustainable Use of Wildlife Unit by the Mexican authorities;
- Creating community guard committees to work in close relationship with authorities;
- Providing research programmes to learn more about amphibian species and their threats; and,
- Ending the commercial breeding of American Bull Frogs and other exotic species and replacing them with local native species.

5.2.3 Madagascar

This case study presents the results of an analysis exploring the trade in amphibians from Madagascar, comparing information contained within two datasets: the UNEP-WCMC CITES database and government data. CITES data were collected June 2006 using the following categories: criteria = live; source = captive bred, ranched, wild caught and F1; purpose = commercial, zoo, scientific. The data analysis was performed on the import data only, due to the caveats highlighted by Carpenter (2003). Malagasy government amphibian trade levels were collated and supplied for the period between 2000 and 2006 by S. Rabesihanaka (2006). Trade network and economic structures were collected from the Ministére des Eaux et Forét and Ministére des l'élevage during the austral summer 2001/02 and taken from Rabemananjara *et al.* (in press).

The CITES database first recorded CITES listed amphibian species being traded from Madagascar in 1994, and between 1994 and 2006 a total of nearly 162,000 individuals were traded in 18 species. Government data for the period between 2000 and 2006 record a total of over 221,000 individuals in 91 species. Species recorded in the trade represented four genera in the CITES database, while nine genera were represented in government data. Malagasy government data has recorded a dramatic, yearly decline over this period in the number of individual amphibians being exported, which is partially supported by the CITES data set (Fig. 5.1).

The CITES data shows that nearly 38% of the trade is accounted for by *Mantella aurantiaca* followed by *M. madagascarienses* (13%) and *Mantella spp* (12%). Similarly, *Mantella aurantiaca* has recorded the highest number of years in the trade (10 years) with four other *Mantella spp* each recording 9 years in the trade. The government data recorded 23 species contributing ≥ 1% towards the over 221,000 total of individuals exported from Madagascar. Six species contributed > 5% totaling nearly 50% of the total number exported, with *Mantella madagascariensis* recording over 14%, *M. aurantiaca* over 9%, *M. viridis* and *M. pulchra* over 6%.

Comparing the CITES and government datasets at the genus level, *Mantella* recorded over 150,000 in both datasets, a magnitude greater than other genera. The genera *Dyscophus* (21,951) and *Scaphiophryne* (26,289) recorded the second and third highest numbers, again, a magnitude greater

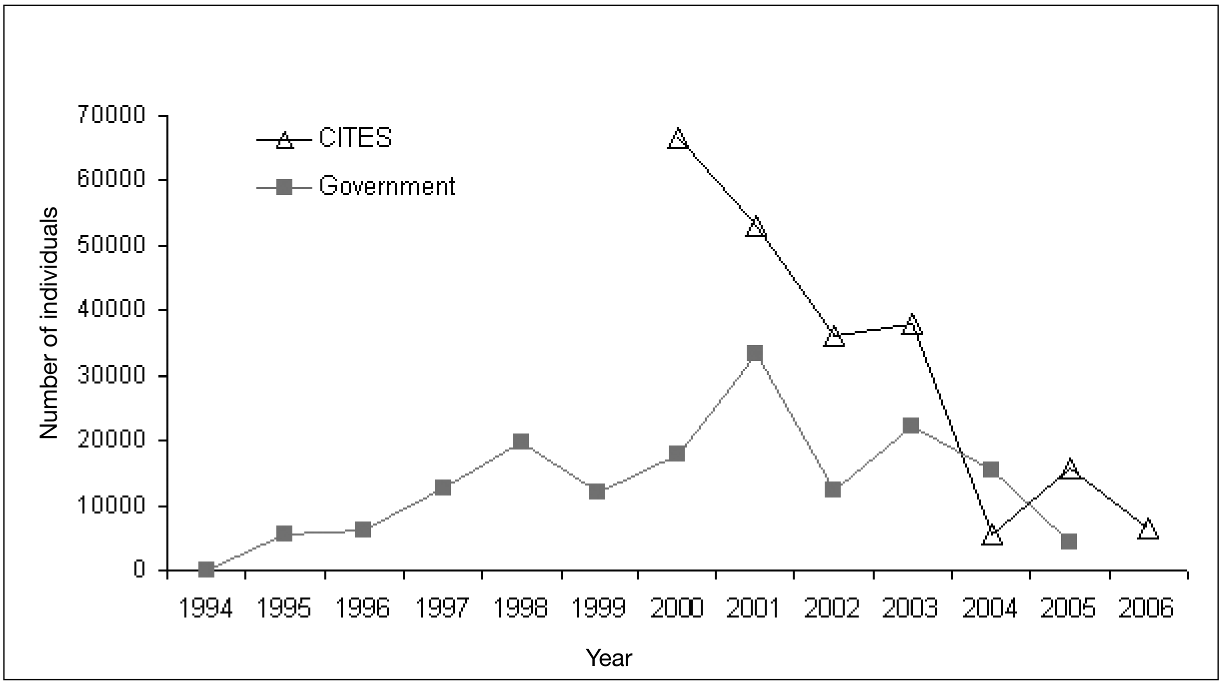

Figure 5.1. Trading trends for the number of individuals of amphibians exported between 1994 and 2006 for both CITES and Malagasy government dataset.

than the following genera, in the government dataset. Since 1997, on average there have been approximately 13 species recorded per year in the CITES data set. The Malagasy government data peaked in 2003 with 48 species being listed as nationally threatened but has continually declined since then, recording a low of 29 species in 2006.

The wildlife trade network on Madagascar consists of three tiers; 1) collector, 2) intermediary and 3) exporter (Carpenter, 2003; Carpenter *et al.*, 2004, 2005; Rabemananjara *et al.*, in press). The income revenue generated from the wildlife trade records the collector as receiving only US \$00.10 per animal, while the intermediary receives greater than 4 fold the collector price and the exporter receives greater than a 26 fold increase on the revenue received by the collector (Rabemananjara *et al.*, in press). The numbers involved at each trade network level were a minimum of 71 importers and 27 exporters. However, the numbers of intermediaries and collectors were unknown.

Using the government data, the total amount of revenue generated from the trade for exporters, using the average price of US \$2.65 per frog, is nearly US \$590,000 for the period between 2000 and 2006. Intermediaries received in total US \$95,000 from the exporters, while intermediaries passed on just over US \$22,000 to collectors for the same period. CITES listed species are, however, more desirable, and assuming a US \$5 per animal at export, this section of the trade would be worth a total of nearly US \$810,000 in the fewer CITES listed species.

The trade from Madagascar in amphibians for the international pet market has, therefore, recorded a fairly consistent level of trade in CITES listed species since 1997. However, Malagasy government data has recorded a dramatic, yearly decrease since 2000. CITES listed species are deemed as more desirable, hence their listing. It was observed that in the government data there were five morphs listed under the single species of *Mantella aurantiaca*. These morphs have been used as species names on CITES permits, in 2000/1, also CITES permits with only the genus name were also observed (A. Carpenter, pers. obs.). Similar mislabeling has also been observed in the chameleon trade (Carpenter, 2003) and was considered a way of circumventing CITES governance. Therefore, this raises concerns over the efficiency of government enforcement of CITES protocol and monitoring of the wildlife trade exported from Madagascar. Therefore, the robustness of the Malagasy government issued data is questionable but is, however, the only official numbers at present.

As reported in the chameleon trade (Carpenter *et al.*, 2005), it is local villagers who often do the collecting hence there is much potential for collectors to exist in nearly every village close to suitable habitats throughout Madagascar. However, it is also presumed that the same declining trend in revenues is operating in the amphibian trade that has been observed in the chameleon trade (Carpenter *et al.*, 2005). Also reported by both Carpenter *et al.* (2005) and Rabemananjara *et al.* (in press), the intermediaries act as 'go-betweens' for exporters, going to the collectors and returning with the animals. Because of the distribution of amphibians, and other species with a market demand, there is much potential for this trade to provide alternative incomes to local people, if managed correctly. However, the sources of income for local people need to be carefully understood before any project seeks to provide an alternative or supplementary income source to villagers, such as the study of northern Malagasy villages reported by Carpenter and Robson (in prep).

A high demand for *Mantella species* was observed in this study, and substitution between the various *Mantella* species appears to be high. It also appears that *M. aurantiaca* has been substituted in the trade by *M. madagascariensis*, indicating that traders could be directed to use alternative species if detrimental harvesting impacts were detected in a population. Worryingly however, there were no known studies investigating the population dynamics of any traded amphibian species, despite this trade existing since the mid-1990s. Therefore, despite knowing what data should be collected and previous studies showing the ways to do this, this is not being conducted on Madagascar. Similarly, there are two alternative genera that appear to be increasing in trade numbers, *Scaphiophyryne* and *Dyscophus*, which also have no monitoring programs in place. This lack of population monitoring is of the utmost concern, as presence/absence data collected in present studies will not indicate any negative harvesting impacts until it is too late. There is an urgent need to conduct studies that investigate species:environment relationships as a top priority, especially for those species recording high numbers in the trade.

Certainly considering that Madagascar is one of the top biodiversity hotspots (Myers *et al.*, 2000) and with predictions suggesting that by 2025 forests will only exist in the most remote parts of Madagascar (Green & Sussman, 1990), novel conservation strategies need to be considered. Projects need to be innovative and assume the synergistic and holistic nature suggested by many (Low *et al.*, 1999; Perman *et al.*, 1999; Brown, 1998; Milner-Gulland & Mace, 1998) rather than the single species or taxon approach.

5.3 Global Actions Required

The purpose of the ACAP workshop on over-harvesting was to establish a harvest management programme, concentrating on 15 countries that appeared to be the focus of the heaviest levels of harvest. The actions needed to address this threat were broadly grouped into six main areas:

5.3.1 Sustainable use

- Study the feasibility and develop sustainable use projects (where the biology of the species permits) of common and widespread species with local communities.
- Determine whether to implement a controlled sustainable trade through a trade quota.
- Form alliances and allocate resources for expanding these actions to other places.

5.3.2 Species Action Plans

- Continually identify endangered species threatened by over-harvesting from the information generated from trade monitoring and the GAA dataset.
- Establish conservation action plans for threatened species based on the most updated information.
- Allocate adequate resources for implementation of such plans in collaboration with relevant local bodies and stakeholders.

5.3.3 Trade monitoring

- Establish national networks in priority countries to monitor trade. This will involve gathering import/export statistics, commercial breeding farm data and regular visits to the food, medicinal and pet markets.
- Establish collaboration with TRAFFIC to monitor the International trade and trade in CITES-listed species.
- Provide data directly to the GAA team for assessment and dissemination.

5.3.4 Commercial breeding/raising

- Determine the feasibility of establishing new breeding facilities by using scientific data and business costs.
- Ensure that commercial captive breeding facilities use only species native to their regions to reduce the risk of the spread of disease and invasive exotics.
- Carefully monitor commercial breeding farms for highly valuable species to prevent wild-caught individuals from entering into the trade.

- Establish operational certification systems and allocate resources to explore how to help bring such conditions into place.
- Channel (wherever possible) the benefits generated from commercial captive breeding operations with a proportion of profits returning to conservation in the wild.

5.3.5 Law & enforcement

- Strengthen enforcement of relevant law and regulations should be strengthened through capacity-building and the input of adequate resources to prevent over-harvesting.
- Clarify the authority responsible for conservation, trade and use of amphibians. Better coordination between government bodies and scientific/conservation organizations is needed for effective enforcement.
- Review national law and regulations to make sure they offer adequate protection to the threatened amphibians.Where inefficiencies exist, arrange capacity building to address these.
- Improve cooperation between countries and operators involved in the cross-border trade to prevent over-harvesting and illegal trade of amphibians.
- List species that are threatened by international trade on the appropriate appendices of CITES so that their trade can be regulated and effectively monitored.

5.3.6 Awareness raising

- Convey the importance of amphibians and the widespread impact of over-harvesting to the general public and those in charge of biodiversity conservation in the priority countries through the local media and publicity campaigns.
- Provide local examples of amphibians that should be used in such campaigns.
- Link the publicity campaign with other themes to give a comprehensive picture of the global crisis of amphibian declines.

5.4 Budget

A five-year budget was developed to implement the above proposed global action plan to counter over-harvesting. It is based on a more detailed budget designed to support similar conservation actions in Mexico (Table 5.6).

Table 5.6 Five-year budget to counter over-harvesting threat

Priority Country Programme	Cost (US$)
Mexico	350,000
China	1,000,000
Myanmar	200,000
Thailand	200,000
Laos	200,000
Vietnam	250,000
Cambodia	150,000
Madagascar	200,000
Cameroon	150,000
Argentina	250,000
Bolivia	200,000
Peru	250,000
Russia and other ex-Soviet Union countries	350,000
Indonesia	300,000
Iran	150,000
International Monitoring	100,000
Total	**4,300,000**

Chapter 6

Evaluating the Role of Environmental Contamination in Amphibian Population Declines

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6.1 Amphibian Declines and the Contaminant Hypothesis

Contamination of the environment represents one of the most pervasive threats to global health and one of the greatest long-term environmental challenges faced by humankind. Pesticides, pharmaceuticals, metals, and other contaminants are being introduced into the environment at such high rates and volumes that even the most remote locations of the planet are now contaminated by oceanic and atmospheric transport (LeNoir *et al.* 1999). The effects of these compounds, even at low levels, can result in endocrine disruption, infertility, genetic damage, increased susceptibility to disease, and death in both humans and wildlife. Exposure of early developmental life stages (including human fetuses) to contaminants can result in malformations, abnormal sexual development, and impaired cognitive ability. Understanding how contaminants, alone or in combination with other environmental stressors, affect environmental and human health is a daunting task. However, because amphibians and mammals share so many cellular and physiological properties that can be disrupted by contaminants, research on the effects of ecologically relevant concentrations of toxicants on amphibians can lead to reasonable predictions about such effects on human health and enhance our understanding of how amphibian communities are influenced by contamination.

From the outset of observed amphibian population declines, contaminants were proposed as a possible causal factor. Widespread use of contaminants after World War II resulted in unexpected negative effects on high-profile nontarget wildlife, which caused declines in some taxonomic groups and risked species extinction (e.g., predatory birds). If we have learned anything from environmental history, it is that contaminants can have pervasive effects and alter food webs in unanticipated and harmful ways. The reality is that organisms in both compromised and pristine habitats appear to be chronically exposed to low-levels of contamination, yet we have relatively little data for amphibians compared to other taxonomic groups (Sparling *et al.* 2000).

One of the complications of assessing the roles that contaminants play in real populations and communities, or in amphibian declines, is that there are tens of thousands of contaminants (Kiely *et al.* 2004; Pimentel 2005) that are purposefully released into the environment or as a byproduct of our industrial lifestyle. Therefore, the scope of the contaminant problem is large and making the links between population declines and this diverse group of factors will be difficult. However, there is sufficient evidence to suggest that contaminants are disrupting systems and they clearly have the potential to contribute to population declines, particularly through interacting with other stressors present in the environment. Some of these contaminants have effects that are well understood, while others are just beginning to be recognized.

It is our goal to understand the factors contributing to amphibian declines and to focus efforts that minimize or eliminate these factors. However, there are a number of reasons why detecting amphibian declines due to contamination may be challenging. First, species often differ in their sensitivity to pollutants. Research has demonstrated amphibian's can be differentially affected by contamination both within and among species (Bridges and Semlitsch 2000, 2001; Snodgrass *et al.* 2004, 2005). Further, variation in pesticide application rate, types of pesticides used, break-down products, and exposure levels fluctuate spatially and temporally. A chemical's persistence and toxicity can also vary depending on environmental temperatures and pH, and by the amount and timing of rainfall in a given year that dilutes or concentrates the chemical in aquatic habitats. Thus, amphibians living in areas vulnerable to periodic pesticide exposure may experience the pulse of exposure in varied contexts. So, making clear links between any particular pesticide or any exposure scenario (e.g., timing of exposure, interval of exposure, environmental conditions) with amphibian declines may be difficult based on field observations alone, because field conditions will almost certainly be different among years.

While pollutant exposure may play a role in amphibian declines, their impact may be tied to the presence of other stressors. Many suggest that multiple stressors are the likely culprit for amphibian declines (e.g., Carey *et al.* 2001; Linder *et al.* 2003). Some evidence suggests the presence of multiple stressors does have a greater impact than the additive effects of each individual stress would predict (i.e., synergistic effects: Little *et al.* 2000; Relyea and Mills 2001; Boone and James 2003). Therefore, if multiple sub-lethal stressors can initiate declines, linking declines with causal factors will be more challenging. Ultimately, linking contaminants to declines in nature may be secondary if experimental evidence demonstrates convincingly the negative consequences of exposure to contaminants to individual amphibians and their populations.

6.2 Effects of Contaminants on Amphibians

Compelling evidence suggests contaminants do affect amphibians at the individual, population, community, and landscape level, and some of this evidence suggests that contaminants could contribute to population declines. While contaminant effects that result in direct mortality would most blatantly cause population declines, contaminants could also affect other responses that could lead to declines. For example, contaminants that lengthen the larval period in the aquatic environment could increase duration of time larvae are vulnerable to aquatic predators and desiccation in drying ponds—both of which may decrease juvenile recruitment and lead to population declines. If contaminants reduce mass at metamorphosis, there could be negative consequences for populations because decreased mass at metamorphosis is negatively associated with overwinter survival, time to first reproduction, and reproductive potential (Smith 1987; Semlitsch *et al.* 1988; Berven 1990; Scott 1994). In this way, negative effects on growth could lead to population declines. Additionally, if species differ in sensitivity to contaminants relative abundance of species could be altered, disrupting subsequent competitive and predatory interactions among species and further contributing to declines of sensitive species. Changes in feeding activity or predator-prey behaviors or changes in development (e.g., metamorphic transformation, gonadal development) could also impact species abundance and contribute to declines. All of these effects have been observed with contaminant exposure, which suggests contaminants could contribute to population declines.

Many contaminant studies with amphibians have focused on effects of individual physiology, behavior, and morphology. Contaminant exposure has been found to disrupt the endocrine system, which could affect development and reproduction. Studies document that gonadal development is altered in the presence of low-levels of contamination (e.g., atrazine: Hayes *et al.* 2002, 2003) resulting in underdeveloped testes and/or hermaphroditic frogs. This suggests that successful reproduction of affected individuals may be impaired or eliminated. Studies have also shown that amphibian metamorphosis is affected by contaminants via altered development through effects on thyroid function (Goleman *et al.* 2002; Crump *et al.* 2002; Fort *et al.* 2004). Behavioral changes in predator-prey interactions (Bridges 1999; Verrell 2000) and in feeding and activity (Bridges 1997; Rohr *et al.* 2004; Hatch and Blaustein *et al.* 2003) can also be altered by sublethal exposures to contamination. In addition, amphibian deformities can result from exposure to contaminants (Hopkins *Et al.* 2000; Rohr *et al.* 2003; Bridges *et al.* 2004; Berube *et al.* 2005), although most cases of widespread limb deformities in nature have been linked to parasite infections (Johnson *et al.* 2003). Further, metabolic costs have been shown to increase with metal exposure (Rowe *et al.* 1998). These studies suggest that contaminants are having unintended effects on individuals that may have population-level consequences.

Ecotoxicological studies with amphibians at the population- and community-level are becoming more common in both the laboratory and in field mesocosm studies (reviewed in Boone and James 2005). Toxicity tests focus on mortality or behavioral responses and are often used to determine lethal concentrations to 50% of the population (LC50) and to set levels where there is no observable effect. Standard toxicity tests show that for some contaminants, amphibians are more sensitive than fish (Birge *et al.* 2000), which are used to establish safe environmental levels for aquatic vertebrates. For other contaminants, amphibians are less sensitive than fish (Bridges *et al.* 2002). Although there are often differences in susceptibility to lethal chemical levels, many environmental chemical levels will be sub-lethal to amphibians. Studies conducted in mesocosms ponds and in experimental wetlands suggest that the sub-lethal effects for amphibians can have consequences for development with some contaminants eliminating or reducing amphibian food resources while others increase resources (Boone and James 2003; Mills and Semlitsch 2004; Metts *et al.* 2005; Relyea 2005a); the effects on the amphibian may depend on which components of their food web is affected. These studies show that survival to metamorphosis can be influenced by larval exposure to chemicals, and that the quality of individuals in terms of time and size at metamorphosis can be negatively affected.

There have only been a few studies examining the relationships between amphibian declines and potential causes on regional scales. Those show an interesting outcome, however. Davidson *et al.* (2001, 2002) showed population declines for numerous amphibian species are significantly correlated with chemicals carried by winds from agriculture in California. Other predictions for declines like increased ultraviolet light or climate change did not explain the observed patterns of declines, although habitat destruction did to some extent for some species (Davidson *et al.* 2002). Subsequent analyses have suggested that pesticides that inhibit acetyl-cholinesterase were most strongly correlated with declines (Davidson 2004). This regional study suggests that contaminants could be playing an important role in declines that occur miles away, although the question of how it affects populations remains to be addressed.

6.3 What We Need to Know about Contaminant Effects on Amphibians

Overall, the evidence suggests that contaminants could compromise amphibian communities. However, we are missing critical information to allow us to fully assess the relative contribution of contaminants to declines. Foremost, interactive effects of multiple stressors—whether involving multiple contaminants or contaminants in the presence of disease, pathogens, climate change, or altered habitats—are vital to understand the susceptibility of amphibians to declines. In nature single factors do not act alone, although most studies do not reflect this. While pathogens or disease may appear to explain some population declines, underlying environmental conditions may set the stage and increase susceptibility to disease. As reiterated throughout this document, understanding the amphibian decline phenomenon is going to require an integrative rather than a simplified "one-solution" approach. Examining the interactive effects of contaminants, disease, pathogens, global change, and habitat alteration will be instrumental to planning mitigation measures to thwart declines.

Additionally, experimental contaminant research has focused almost solely on the aquatic life stage for amphibians (but see Hopkins *et al.* 1997, 1999; Laposata and Dunson 2000; James *et al.* 2004a, 2004b; Boone 2005). While there is some reason to believe that the larval stage may be the more sensitive than the terrestrial stage, contaminants could have effects that influence populations in the terrestrial environment. For instance, James *et al.* (2004a) found that exposure to cadmium contamination in sediments during hibernation increased the mass lost and decreased survival of juvenile American toads, suggesting terrestrial exposure could impact population dynamics. Additionally, work by Hayes *et al.* (2002, 2003) demonstrated that changes in gonadal development from sub-lethal herbicide exposure of tadpoles resulted in developmental changes in gonads, which could reduce fertility and lead to population declines. Thus, exposure to contaminants in both the aquatic and terrestrial life stages may impact populations, and we have very limited data focusing on the terrestrial stage or interactions between aquatic and terrestrial stages.

Further, while results suggest that contaminants can affect endpoints that appear to be important to population persistence, like endocrine disruption, fertility, hatching success, survival to metamorphosis, and behavioral changes, we do not understand how contaminants may influence populations through time at multi-generational scales. For instance, unless a pesticide (or an interaction of a pesticide with another factor) completely eliminates a species by reproductive failure, we do not currently have enough data to make reasonable predictions about a population's persistence because contaminant effects could be subtle (as previously discussed). While reduced hatching success or reduced survival to metamorphosis appear negative (and may well be), we do not know what the long-term impact on the population will be if some individuals continue to reach metamorphosis and maturity. Many species of amphibian may be adapted over evolutionary time to deal with periodic bouts of reproductive failure as is suggested by long-term field studies (e.g., Semlitsch *et al.* 1996). Therefore, if contaminant exposure varies in time and space, then observing the outcome will be confounded by natural population variation and variable exposures over long time periods. Few studies have addressed physiological or genetic adaptation to long-term chemical exposure, or how adaptation to a chemical stressor may influence population persistence or make individuals vulnerable to other factors (e.g., Semlitsch *et al.* 2000).

Although much has been learned in recent years about the effects of a few contaminants on amphibians (e.g., carbaryl, atrazine, coal combustion wastes), little is known about the effects of most other common pollutants on amphibians. Beginning to assess ecological changes that ensue with exposure to representative chemicals from contaminant classes with particular modes of action would be a way to begin. Other groups of contamination resulting from industry by-products, as well as human and other animal waste products (pharmaceuticals, sewage by-products), pose potential threats to wildlife and have been virtually ignored. It is difficult to say which contaminants may pose the greatest risk to any organisms, particularly amphibians given that they are not routinely used in toxicology testing. The US Environmental Protection Agency collects information on the quantities of registered pesticides that are sold on the US and World market. From this information, it is known that throughout the world, herbicides are the most commonly applied pesticide, followed by insecticides and fungicides (Kiely *et al.* 2004). Therefore, based on sheer volume of pesticides applied, herbicides could pose the greatest threat. While herbicides typically have modes of action that are unlikely to affect animals, the herbicide atrazine has been found to disrupt the endocrine system (Hayes *et al.* 2002, 2003), the carrier of the herbicide glyphosate has been found to have toxic effects on amphibians (Howe *et al.* 2004; Relyea 2005b), and herbicides can reduce the food base of the community (Diana *et al.* 2000; Boone and James 2003). Therefore, herbicides may have important negative effects on amphibian populations that need to be examined. Insecticides have modes of action that are likely to affect amphibians and other organisms in their food web; so, while a lesser amount of active ingredient is applied, the effect could be more (or equally) detrimental.

We also have very poor information for the types of contaminants and combinations of contaminants that amphibians are exposed to in nature. More research is needed to address this problem. Additionally, research on ways to minimize movement of chemicals through use of "safe" chemical application or "best management practices" like habitat buffers that reduce movement could benefit all non-target populations that are affected by contaminant exposure.

Current research needs include examining worldwide patterns of contamination (and other causal factors) and declines (e.g., Davidson *et al.* 2002), understanding the population-level consequences of all the major chemical classes of contaminant exposure over time and in the presence of other stressors, examining the evolutionary consequences of contaminant exposure, and determining ways to minimize movement of contamination at regional or global levels.

6.4 Proposed Actions

Because evidence suggests that contaminants in the presence of other stressors have a strong potential to impact amphibians negatively, action should be taken to protect non-target populations from contamination. Efforts could be divided into "emergency," "short-term," and "long-term" actions that would be protective of amphibians and the communities they live in. Sites where declines are occurring should be evaluated for environmental contamination that may be present through direct application or movement through air or water; this survey data would help us determine if dangerous levels of contamination were present, which may necessitate emergency clean-up action, and would establish chemicals present to guide pertinent research efforts (e.g., interactive effects of contamination and pathogens). Short-term goals should focus on examining the relationships between declines and potential causes (e.g., Davidson *et al.* 2002); evidence that contaminants routinely are correlated with declines would offer a "weight of evidence" to support the relationships between declines and contaminants, which would justify regulating contaminant application more rigorously. Long-term goals should focus on experimental studies that lead to cause-and-effect relationships to further influence regulatory standards in ways that have meaningful impacts on organisms, as well as further our understanding of how contaminants are influencing community regulation of amphibian communities.

To achieve these goals, an ecotoxicology consortium should be established of internationally recognized amphibian biologists working at the forefront of ecotoxicology. We envision a group of approximately ten people that are working on a broad range of topics pertaining to declines and dedicated to developing solutions. An international director would be assigned to lead the group on scientific matters and decisions. The group would establish research objectives and fund competitive research projects annually; prioritize contaminants, species, and locations of concern; work with regulatory agents to make amphibian testing part of federal protocols; and coordinate research efforts at international centers. Additionally, this group would foster research that synthesizes the available scientific and technical literature (including structured reviews or meta-analyses) as a means of objectively assessing research priorities in the field. To develop innovative ideas for solutions, we propose to meet annually with research scientists and officials representing a diversity of federal agencies in countries where pollutants are suspected or demonstrated to negatively impact amphibian populations (e.g., national agencies equivalent to the US Environmental Protection Agency, US Geological Survey, US Forest Service, US National Parks, US Bureau of Land Management). Additionally, these meetings would help to assess current research results, develop and prioritize research objectives, and to discuss potential solutions. The consortium would issue an annual call for proposals that meets our research and solution objectives. Research proposals would then be reviewed for funding by the consortium and ad hoc reviewers based on meeting research objectives, level of innovation, and the potential to lead to solutions. Funding would be especially critical for large-scale (e.g., whole-ecosystem manipulations), interdisciplinary (e.g., biology integrated with economics/sociology), or high-risk projects that would not normally be considered by traditional funding agencies (e.g., US National Science Foundation). Additionally, research projects in biodiversity hotspots or projects dealing with faunas and biomes that are underrepresented in ecotoxicological research and/or of conservation concern will also be a priority. We propose that one to three year projects be reviewed each year for funding at an annual meeting by the consortium.

Additionally, we propose establishing centers on each continent (in association with centers being developed for disease research and captive breeding) to address regional contaminants of concerns on amphibians. Potential sites would take advantage of existing infrastructure. Possible sites in North America include the University of Georgia's Savannah River Ecology Laboratory in Aiken, SC, and U.S. Geological Survey's Columbia Environmental Research Center along with the University of Missouri—Columbia in Columbia, MO; both sites have researchers with expertise in environmental toxicology and are locations where research with amphibians and contaminants is currently being conducted. A strategic site in South America includes the Environmental Management Unit of the University of São Paulo in São Paulo, Brazil. This site combines technical infrastructure, regional and international accessibility, and a strategic biogeographic location in the central corridor of the Atlantic Forest, a hotspot where amphibian declines have been detected.

6.5 Conclusions

In conclusion, we propose that innovative ecotoxicological research incorporating the stresses associated with disease, climate change, and habitat alteration will be the cornerstone of understanding and preventing amphibian population declines. Research is needed that goes beyond traditional toxicity testing by understanding complex chemical mixtures in complicated natural environments. Reducing use and spread of contaminants will minimize the risk to all populations; however, providing direct links will be pivotal in convincing citizens and governments to support limitations of contaminant application. Addressing research priorities outlined here will provide the data needed to protect communities in nature, will ultimately be protective of human life as well.

6.6 Budget

Table 6.1. Summary of estimated costs to implement actions outlined, in US $, for each year up to five years.

Activity	Initial	Year 1	Year 2	Year 3	Year 4	Year 5
6 International Centers						
Building/renovation	100,000	600,000	0	0	0	0
Start-up for machinery	100,000	600,000	600,000	600,000	600,000	600,000
Chemical Analyses	1,000	600,000	600,000	600,000	600,000	600,000
Staff	100,000	600,000	600,000	600,000	600,000	600,000
Research						
Competitive Grant (NSF model)*	500,000	3,000,000	6,000,000	9,000,000	9,000,000	9,000,000
Consortium Annual Meeting	50,000	50,000	50,000	50,000	50,000	50,000
Total		5,350,000	7,210,000	10,210,000	10,210,000	10,210,000
Five-year Total						43,190,000

* Some funds allocated for graduate student seed grants funds.

Chapter 7

Captive Programs

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7.1 Introduction

In the face of overwhelming and sometimes urgent threats to many amphibians, such as disease or habitat destruction, the only hope in the short-term for populations and species at immediate risk of extinction is immediate rescue for the establishment and management of captive survival-assurance colonies. Such programs are not the final solution to conservation of any species, but in some circumstances may be the only chance to preserve the option for eventual recovery of a species or population to secured habitat. The captive-assurance strategy has to be implemented as part of an integrated plan that includes research on amphibian biology and disease, development of improved husbandry, training and building capacity in range countries, and mitigation of threats in the wild. The existence of the captive assurance colonies will facilitate many of the goals of other branches of ACAP, including basic research on amphibians and their diseases. Captive programs do not replace important programs related to habitat preservation, control of harvesting, climate change, and ecotoxicology, but instead provide options and resources to enable survival of some species while these research programs proceed.

7.2 Actions

While there is consensus that captive programs will often be an essential component of integrated amphibian conservation plans, the traditional zoo/aquarium/garden infrastructure cannot currently accommodate a program on the scale required. A global network of captive breeding programs that are explicitly linked to conservation and research programs—The Amphibian Ark (AArk)—has therefore been formed to implement the ex situ component of ACAP. Activities will be implemented in four phases:

7.2.1 Information gathering and emergency collections; preliminary captive operations

Operating in response to recommendations from local biologists, national governments, and the various ACAP research branches, rapid-response teams would travel to sites predicted to suffer catastrophic losses to implement pre-emptive collections of animals that will form the basis of captive programs. A prototype of such a program has been used effectively to rescue the frog fauna of a site in Panama (see www.saveafrog.org).

7.2.2 Establishment of captive operations in the range countries

Central to the long-term success of a captive program is the establishment of captive operations in range countries. Infrastructure for such facilities may be reasonably established with portable, modular units (e.g., modified shipping containers) or by simply adapting local warehouses or houses or local infrastructure such as botanic gardens, university biology departments, industrial or government complexes that are either under-utilized or purpose adapted for the management of amphibian species. Local biologists or citizens must quickly be identified, hired, and trained in basic amphibian husbandry. A steady program of internships in established amphibian facilities in other countries will be critical to maintaining intellectual and practical capacity at range-country facilities. Close contact and communication among all facilities in the network must be maintained by a global supervisory staff. Range-country programs will operate in native languages, and will be aimed to ensure that operative protocols are matched to local conditions, culture, and infrastructure.

7.2.3 Research and long-term maintenance of captive operations.

In addition to securing captive colonies in small, modular facilities, back-up populations will be secured in larger, multi-species facilities that provide for efficient care, breeding, and research on many species. These larger facilities may be in the range country and/or in facilities and programs outside the range country. Furthermore, these facilities will provide the capacity and facilities for research and implementation of cryobanking of gametes of threatened species, thereby serving as an additional safeguard for species, populations and specific genetic lineages (Appendix A). Note that such cryobanking of gametes complements the cryobanking of cell cultures discussed in Chapter 11.

7.2.4 Providing animals for research and reintroduction programs

The captive colonies will produce the animals needed to meet long-term research needs and to provide animals for the ultimate goal of reintroduction to natural habitats.

7.3 Challenges and Opportunities

At present, our knowledge of the status of amphibians is unequal across the globe—some countries have already been devastated, others are zones of active decline, some species and regions apparently are unaffected, and many are sorely data deficient. In addition, capacity to implement major conservation actions varies widely among affected nations. Consequently, the need for, and scale of, captive operations in any particular area will differ according to regional conditions. Since regional capacities to implement captive programs will also be variable, the starting point in the process will differ among regions and only ongoing surveys and research will identify the actual numbers of species that will require a captive component to their overall conservation plan.

An advisory board serving the captive program would work with regional biologists and other experts to lead the difficult task of prioritizing species and sites for inclusion in captive programs or for ex situ support of in situ field research and conservation initiatives, and also for defining timely exit strategies for each program. This advisory board would also conduct regular evaluations of each program to determine its relative success or failure.

Captive programs would also have as their focus to lead research activities (e.g., pathology, immunology, evolution of resistance), as well as traditional activities of captive breeding and husbandry. Further research into emerging infectious diseases and climate change is crucial to the success of the overall program, as are studies of basic amphibian ecology and natural history. As many of the necessary research programs in fields such as immunology and pathology will require captive colonies, the AArk network must work closely with scientists from those disciplines to achieve the overall goal of conservation of amphibian biodiversity.

7.3.1 Logistics, infrastructure, diplomacy and capacity

There are formidable bureaucratic obstacles associated with collecting large numbers of animals (and, in some cases, moving them across national borders) on a short time-scale. This reality speaks to the need to emphasize that development of range-country programs is an essential focus of this program, and that exportation of animals will only be conducted when legally possible and no other option is possible. Equally critical will be the necessity of identifying and hiring local or national persons to manage regional operations and facilities. Range-country programs would be charged with evaluating species and regions to be considered, making recommendations to the governments that have ultimate authority to determine that a captive program will be implemented for a species, and with managing legal affairs related to collection and import/export of animals among facilities in the network.

The physical structures needed for captive breeding programs will range from simple, temporary facilities that can be erected adjacent to the species' habitat, to large, specialized multi-species facilities at remote locations. Several models for small, modular captive breeding facilities—e.g. climate-controlled shipping containers—have been developed and tested in Australia, and a model program using available buildings in local villages has been implemented in Panama. Implementation of range-country programs will require training of personnel at each site in basic husbandry, management, and research techniques. Thus, capacity-building programs will include internship and personnel exchange opportunities to disseminate and maintain expertise among the global network of facilities.

Partnerships with zoos, aquariums, fish hatcheries, industry, government and university facilities and botanic gardens will assist with funding, implementation, staff training, and sometimes the hosting of captive breeding centers—both in range countries and external to range countries.

7.3.2 Priority science gaps for research and future focus

Many of the species in need of urgent implementation of captive programs have never before been maintained in captivity. Thus, most programs will face substantial challenges related to basic husbandry and reproduction at the outset. While these captive colonies will represent a crucial element of the overall survival plan for a particular species, they will simultaneously provide important opportunities to conduct research related to disease susceptibility, management and treatments, reproductive biology, and tolerance of environmental elements related to climate and toxins. For example, while various ACAP groups work to better understand the biology, pathology, and potential to control chytrid fungi, captive programs must work with geneticists and immunologists to research the potential for populations to evolve resistance to the fungal pathogen.

7.4 Budget

A dedicated infrastructure of staff and facilities will require long-term commitments of reliable financial support. The model and budget proposed here has been based on costs and estimates derived from sources in USA, and experiences in Latin America and Australia. As this program is intended to be global in scope, we anticipate all cost estimates will vary geographically. The program budget uses these sample costs to estimate average costs per species. The total cost of the captive breeding component of ACAP will depend on the number of species for which it is determined that captive maintenance is a required component of their conservation action plan. Note that the separate budget for the cryobanking of gametes (Appendix A) is combined with the following captive breeding budget within the Executive Summary to ACAP.

7.4.1 Per Species Model

	US $
Species Preservation in Captivity (2 separate secure populations)	
Amphibian Captive Facility 25,000 x 2	50,000
Maintenance and overhead 10,000 x 2	20,000
Amphibian Keeper Staff 1 per population x 2	50,000
Year one Total per species:	**120,000**
Fulltime coordinator staff (4), incl. benefits & travel	400,000
Six training workshops/year	100,000

7.4.2 Year one example:

*100 Species (@ 120,000) 12,000,000	
Coordinating Staff	400,000
Worskhops (6/yr)	100,000
Total	**12,500,000**

7.4.2 Four years on-going maintenance:

Maintenance of 100 species (@ 20,000)	8,000,000
Amphibian Keeper Staff (@ 50,000) 20,000,000	
Coordinating Staff	400,000
Worskhops (6/yr)	100,000
Total	**28,500,000**

Grand Total 100 species for 5 years set and maintenance 41,000,000

***Arbitrary number of species for illustration purposes. For example, 20 species from each of 5 critical areas.**

Appendix A
Genome Resource Banking

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Introduction

Populations of amphibians are declining globally at an alarmingly rapid rate (Stuart et al., 2004). Unless a large-scale, multidisciplinary and integrated conservation effort is mounted soon, it is likely that several hundred or even thousands of species will be lost forever (Butchart et al., 2005; Mendelson et al., 2006; Stuart et al., 2004). In response to this crisis, biologists and zoo professionals have endorsed the establishment of 'insurance colonies' for selected amphibians, especially as a hedge for populations and species at immediate risk of extinction. However, in any *ex situ* conservation effort, the goal is not simply to preserve a species, but to ensure that the offspring produced are genetically valuable, viable and capable of contributing to self-sustainability (Wildt et al., 1997). Typically, most captive breeding programs strive to maintain 90% of all existing gene diversity over a period of 50 to 100 years. Achieving this goal is influenced by number of founder animals in the managed population, reproductive success in captivity, generation interval and offspring survival. Unfortunately, managed populations have often failed due to poor reproductive success and/or lack of sufficient founders to maximize genetic integrity. One vital tool for preserving the genetic signature of a species, maintaining gene diversity, increasing the founder base and improving overall reproductive success is the intensive management and use of biomaterials, including germplasm, embryos, tissues, blood products, and DNA. To be effective requires specialized technologies and systems to preserve this material – the formation of a Genome Resource Bank (GRB), an organized repository that, if used, judiciously, can serve as a second line of defense against species extinction.

A GRB in combination with assisted reproductive techniques, such as artificial insemination and *in vitro* fertilization, offers: 1) a viable, cryopreserved, but alive resource of all existing genes that can be used for reproduction and basic/applied research; 2) easy, economical movement of genetic material between populations and across borders and/or between captive and wild populations; 3) enhanced reproductive success in animals that fail to breed naturally; 4) the ability to minimize gene loss via genetic drift while extending the generation interval and the ability to manage the reintroduction of "original" genes over time; 5) minimal space requirements; and 6) a means to enhance scientific research pertaining to other scientific disciplines, including systematics, phylogenetics, health and disease exposure (Wildt et al., 1997).

Broad Goals and Implications

Based on the overall benefits that could be derived, a GRB program could be a valuable asset to amphibian recovery efforts, especially in the context of captive breeding programs and field efforts where substantial numbers of individual animals are being handled and/or captured. For example, as field surveys are being conducted to assess population status and health, it could be relatively easy to collect biomaterials that could form the foundation of a GRB. This could include short-term periods in captivity where animals become germplasm donors before being released back into nature. Such efforts would capture existing genetic composition of wild founders that, in turn, could provide invaluable biomaterials for sustaining or re-invigorating *ex situ* insurance collections. Simultaneously, the evaluation of reproductive status of field specimens will provide important indicator information on the potential impact of contemporary environmental perturbations, from stress to contaminants exposure to inbreeding depression. Virtually nothing is known about the reproductive mechanisms or even basic physiology of most amphibian species. By developing protocols for measuring reproductive traits in the context of morphometric and physiologic measurements, such efforts will build a foundation of basic knowledge that is likely to have practical application to the management and conservation of amphibians *ex situ* and *in situ*.

The highest priority and short-term goal is to develop standardized and non-invasive methods for the collection, assessment and storage of germplasm (sperm, oocytes or embryos) from a variety of amphibian species. When available, this information could be used practically to maximize genetic diversity and, ultimately, contribute to conserving critically endangered amphibians through enhanced captive breeding and management. We also anticipate that such data would be predictive of the extent of impact of environmental change (disease, climate change, contaminants and human encroachment) on fitness of amphibian populations.

Key research actions: priority science gaps and future focus

The IUCN's Global Amphibian Assessment (IUCN, 2006) recognizes more than 5,900 species of amphibians, most of which have never been studied. Traditional amphibian research has focused on only a few species used as models for basic biological and/or biomedical research (e.g., *Xenopus laevis* and the *Ambystoma* salamanders). In the current amphibian decline crisis, this lack of fundamental knowledge limits the ability of scientists to assess population health and survivability. In many cases, even the basic life stages of amphibian species under threat are unknown. A comprehensive genome resource banking effort, therefore, will require both fundamental knowledge as well as specialized information on cryosensitivity of germplasm and other biomaterials. This will ensure that the living material is safely stored, biologically viable and capable of being used for propagation and maintaining the genetic integrity of populations and species. To initiate this program, we will select a few model species representing the three major orders of amphibians: 1) frogs and toads, 2) newts and salamanders and 3) caecilians. These models also will be chosen to represent the major types of reproductive strategies in amphibians (e.g., internal versus external fertilization, aquatic versus terrestrial egg laying). A comprehensive research program then will be initiated to generate the fundamental knowledge in basic biology for each species (timing and type of life stages, types of reproduction, basic endocrinology, behavior) while at the same time determining methods of collecting and freezing genomic specimens, primarily spermatozoa. The combined results then can be used in applied studies to demonstrate the efficiency of using frozen spermatozoa for propagating (and ultimately helping to conserve) amphibians. Of particular interest will be answering the question – how conserved are mechanisms across species and taxa. Finding similarities in biological mechanisms will suggest rapid practical application to endangered counterparts; finding diversity will emphasize the need for intensive species-by-species attention prior to large-scale captive propagation efforts.

Spermatozoa have been collected from amphibians after an intraperitoneal injection of gonadotropins (Kouba et al., 2003; Licht, 1973; McKinnell et al., 1976; Obringer et al., 2000; Waggener and Carroll, 1998; Waggenner and Carroll, 1998). However, this technique has the disadvantage of contributing to abdominal adhesions, bowel puncture or introduction of infectious agents. A non-invasive technique for inducing spermiation has been reported for the American toad (*Bufo americanus*) and the Wyoming toad (*Bufo baxteri*) (Browne et al., 2006a; Browne et al., 2006b). Here, luteinizing hormone-releasing hormone (LHRH) alone or in combination with human chorionic gonadotropin (hCG) is applied to the abdominal seat region or fed concealed in a meal worm. Results have suggested that spermiation occurs in a high proportion of males within 4 to 5 h of treatment. Amphibian spermatozoa also have been cryopreserved using various cryoprotectants, including dimethylsulfoxide, glycerol and sucrose (Beesley et al., 1998; Browne et al., 2002; Browne et al., 1998; Costanzo et al., 1998; Michael and Jones, 2004).

Our general aim is to establish and sustain an active genome resource bank that can contribute to conserving rare amphibians. To achieve this aim, the immediate research objectives are to:

1) determine optimal model species that represent a range of amphibian orders and then, through systematic studies, determine fundamental reproductive strategies for each.
2) develop safe, non-invasive methodologies for recoverying viable sperm.
3) increase our understanding of cryosensitivity of amphibian spermatozoa.
4) develop ‘field-friendly’ sperm cryopreservation technologies and tools for assessing the viability/functionality of thawed sperm.
5) establish methods for recovering viable spermatozoa from fresh carcasses.
6) demonstrate the biological competence of cryopreserved spermatozoa through the production of healthy offspring.
7) conduct the necessary computer modeling required to determine the optimal number of individuals to be banked.
8) implement and maintain an inventory and database for effective management of cryopreserved samples.
9) Increase scientific capacity in-country through training to routinely allow large-scale and safe collection and cryopreservation of germplasm from free-living and captive amphibians.

Our immediate logistical objectives are to:

1) explore the possibility of establishing satellite (back-up) locations for storage of bio-materials.
2) establish a funding mechanism to manage the cryopreserved samples (including personnel, equipment, liquid nitrogen, storage space).
3) generate a web-site comprised of information pertaining to species biomaterials within the GRB.

Logistics

Most of the research objectives could be met, at least initially, by competent scientists working in (or with) zoological collections. These individuals must have a strong commitment to capacity building, especially the training of counterparts in range countries that have high priority species requiring attention. Ideally, studies would begin in North American zoos and, once the models were identified and research colonies developed, studies would begin, preferably with a senior scientist mentoring multiple post-doctoral fellows, graduate or undergraduate students. Some of the trainees eventually must come from range countries where there is an eventual goal to develop research/propagation programs for high priority species. This will require the development of laboratory and *ex situ* breeding facilities.

Budget

See Table 1.

Table 1. Five-year budget for implementing the actions outlined.

Component	Justification	US$
Research: Funding for 3 post-doctoral or graduate fellows to develop methodologies for improving spermiation induction and sperm cryopreservation	Post-doctoral fellowships is estimated to cost $38,000/year/person plus $1,800 in health benefits, $2,000 in travel and $5,000 in research supplies. Duration of fellowship, 3 years.	421,200
Salary for in-country research staff (3 people) to collect, bank and manage the GRB	Salary $25,000/year/person plus travel $2,000/year/person for 5 years.	405,000
Infrastructure development in range countries	Microscopes (2; $12,000); liquid nitrogen tanks ($6,000); liquid nitrogen $1,000/year; dry-shippers ($1,500/shipper = 2); disposable supplies $6,000/year	68,000
Other supplies	Disposable supplies, cryodiluents, dyes and media; $20,000/year	100,000
Total for 5 years		**994,200**

Chapter 8

Reintroductions

R. Griffiths, K. Buhlmann, J. McKay,
and T. Tuberville

8.1 Introduction

Reintroduction of animals to the wild has frequently been promoted as the primary reason for breeding animals in captivity. However, captive breeding may contribute to conservation through actions that do not involve reintroduction (e.g., education, research) and reintroductions do not necessarily involve a captive component. Indeed, for many amphibian species, reintroductions may be achieved more efficiently, more safely and more cost effectively if they do not involve a captive breeding component. Simple translocation of spawn or tadpoles, for example, can be an effective tool in species recovery. Where high levels of spawn or tadpole mortality are prevalent, head-starting tadpoles by raising them beyond the stages at which they are vulnerable to competitors, predators or other threats may also be preferable to captive breeding. Nevertheless, there are many issues that need to be carefully considered and addressed when a reintroduction is planned or carried out. The IUCN (1998) guidelines for reintroductions provide a framework for the protocols to be followed for amphibians, but may need modifying in view of species-specific requirements or linkages to other themes within ACAP.

Many species are likely to recover on their own following mitigation of the threats coupled with habitat management, restoration or creation. Indeed, natural recolonization is likely to be more effective in terms of establishing viable populations, as well as logistics and costs. If natural recolonization is not possible because the restored habitat is isolated, consideration needs to be given to whether the area can support a viable population (or metapopulation) even if a reintroduction takes place. Reintroduction should therefore only be considered as an option where these mechanisms are deemed insufficient for ensuring species recovery on their own.

Whether or not they involve captive breeding, reintroduction programs for amphibians are at an early stage of development, and it will be many years before we can make unqualified judgements concerning their effectiveness as a tool for conservation. Certainly more science is needed, but given the current biodiversity crisis, we cannot wait for all the necessary hypotheses to be rigorously tested before acting. Adaptive management—which relies on continuous review and refinement of program protocols based on prior experience—will therefore always be an integral part of amphibian reintroduction programs, and of conservation programs in general.

8.2 Selecting Species for Reintroduction

Although a large number of species are recommended for reintroduction within the Global Amphibian Assessement (GAA), the selection of these species appears to be rather arbitrary and not based on objective criteria. There appears to be variation between regions in the tendency to recommend species for reintroduction, and this may reflect regional variation in expertise and personal interests rather than real needs for reintroduction. It is therefore essential that species are carefully appraised for their suitability for reintroduction.

The following criteria provide guidance for evaluating whether a species is suitable for reintroduction.

8.2.1 Status and distribution of the species

Without this information, it is difficult to make any objective recommendations for conservation or assess whether reintroduction is appropriate. Priorities for reintroduction should focus on globally threatened species, although locally threatened species may also be considered when they are of local political or cultural importance.

8.2.2 Reversibility of threats

The most successful animal reintroductions have usually involved those species that have threats that are easily neutralized (Griffith *et al.* 1989; Caughley 1994; Wilson and Stanley-Price 1994). Threats that are more likely to be reversible are often those associated with direct persecution, pollution and those that can be realistically addressed using legal, political or cultural processes that are enforceable. It is often easier to reverse threats in clearly delimited geographical areas, such as islands, than it is in habitats that grade into each other. One problem facing amphibians is that the threats that they face are complex, often synergistic, and not easily reversed (Beebee and Griffiths 2005). The reversibility of threats should therefore influence which species are considered for reintroduction programs. Reversing localized agents of decline, such as introductions of fish or other predators, is likely to be more feasible than reversing global threats such as climate change and increased UV-B.

8.2.3 Life history

Species in which certain life stages can be safely collected and translocated without detriment to the donor population will be most suitable for reintroductions. Such species will usually be those that have high fecundity and robust eggs, larvae or metamorphs that can be transported easily. Donor populations of species that display clear density dependence in larval development and survival are less likely to be impacted by the extraction of animals for translocation than populations that display other forms of population regulation.

8.2.4 Geographical priorities

Geographical priorities may be associated with priority areas for conservation, or areas where the political or logistic support is likely to increase the chances of success of a reintroduction. Most reintroductions carried out to date have been in temperate areas, rather than in those areas that support high levels of amphibian diversity. Careful consideration therefore needs to be given to balancing priorities between those geographical regions that are low in biodiversity but rich in expertise and infrastructure, and those areas poor in expertise and infrastructure but rich in biodiversity.

8.2.5 Taxonomic priorities

Monotypic genera or families, members of ancient lineages or taxa that are otherwise poorly represented in conservation programs may be considered a priority in some circumstances. Where expertise and knowledge has been previously gained on a widespread or non-declining species, it may be cost-effective to consider closely related, threatened species for reintroduction as these may benefit from the existing knowledge base.

8.2.6 Wider biodiversity considerations

When a species is part of an ecological community or natural system that is of wider biodiversity interest, it may be considered a priority. Such species may play an important role in maintaining community structure and thereby influence other aspects of biodiversity.

8.3 Actions to Execute a Reintroduction

8.3.1 Publicity, public relations and information

These will be achieved by timely press releases, information leaflets, website information, T-shirts, post cards etc. In some cases it may be possible to develop nature tourism and possibly other economic incentives based on the species concerned. These actions should mobilize public support and consolidate political—and possibly financial—backing for the project.

8.3.2 Pre-release assessment of the wild populations

The status and distribution of the species will be assessed by a combination of interrogation of existing sources of information (e.g., GAA, local atlases etc.) and field survey. Refinement of existing survey methodologies may be required as an adjunct research activity to allow this. Priority species will be those that have undergone clear contractions in historical range, and which would be unable to re-establish functional populations (or metapopulations) within that range without reintroduction. Introductions to areas outside the historical range will usually be discouraged, although climate change data may suggest that unsuitable areas outside the natural range may become suitable sometime in the future. Equally, restocking (or supplementing) existing populations carries disease and genetic risks (see below) and should not be considered unless numbers have fallen below those required for a minimum viable population and the associated risks have been assessed.

8.3.3 Applied ecological research on life history and habitat requirements

Basic population demographic data on the species will 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 the reintroductions. 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 reintroduction succeeding (see below).

8.3.4 Threat mitigation, habitat management, restoration and creation

The threats leading to the decline or extinction of the species will be evaluated and neutralized following the protocol described by Caughley (1994). It is unlikely that some important threats to amphibians (e.g., climate change, UV-B, etc.) can be neutralized, at least in the short to medium term. In such cases, reintroduction is unlikely to be a sensible option.

Following the assessment of habitat requirements, potential reintroduction sites will be evaluated for management requirements. The program of habitat management will involve maintaining or enhancing existing areas, restoring areas that still exist but have become unsuitable and creation of new habitat where appropriate (or a combination thereof).

8.3.5 Population viability analysis, release protocols, and strategic recovery plan development

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 (Akcakaya *et al.* 2004). These targets should then be embraced within a staged planning process, with interim milestones if necessary to monitor progress as the project develops. Knowledge of the life history of the species should be used to determine appropriate targets and time frames for success. EU legislation requires member states to maintain—or restore to—'favorable conservation status' those species of community interest, and this is being used as a generic target in many species recovery programs (although explicit definitions of this term may vary from species to species, and region to region).

The reintroductions will involve the release of eggs, larvae and/or metamorphs, as previous reintroduction programs have shown that using these stages is most likely to lead to success. However, further research is needed on release protocols, (e.g., the relative proportions of the different stages, 'soft' vs. 'hard' releases, trade-offs of captive vs. wild stock, applicability of head-starting technologies). The reintroductions will therefore serve as ecological experiments for testing hypotheses concerning these issues, and protocols will be refined accordingly.

An appropriate organizational infrastructure needs to be established to ensure the success of the program. This will invariably require the cooperation of a wide spectrum of stakeholders ranging from local communities to government officials. There may be legal obstacles associated with the release of organisms into the wild that need to be overcome. Effective lines of communication need to be established, language barriers overcome and transparent mechanisms for resolving differences of opinions established.

8.3.6 Risk analysis

The movement of living organisms from one place to another carries various risks. These risks may be genetic, ecological or socio-economic. Genetic risks are associated with the release of maladapted animals into an area. Donor populations will be screened for any potential problems associated will possible maladaptations or inbreeding. This will be combined with a landscape level analysis of the release site to ensure that the released population will not suffer from any genetic problems as a result of habitat isolation in the future. There may also be concern over the release of animals whose taxonomic relationships are unresolved. Linkage with the ACAP Systematics Working Group will be maintained to resolve any issues in this area.

Ecological risks embrace issues associated with the inadvertent transmission of disease or other organisms. Apparently benign organisms may have unforeseen impacts on food chains when transmitted to new environments. Protocols will therefore be in place to minimize the risk of transmission of propagules of potentially invasive species. Comprehensive health screening will be carried out on 1) animals from the donor population (captive or wild); 2) all amphibian species present at the release site. The protocols will follow those established by the ACAP Disease Working Group (See Chapter 4). Socio-economic risks are associated with impacts on the livelihoods of local people. If the reintroduction results in the exclusion of people from traditional areas or ecological impacts that impact on agriculture or other income-generating activities, there may be ramifications for its likely success. Surveys of attitudes towards the reintroduction within local communities will therefore be carried out and any conflicts of interest resolved.

8.3.7 Post-release monitoring

Many amphibian species have cryptic life styles that render them extremely difficult to monitor. Consequently, research on the refinement of monitoring protocols will inform the design of post-release monitoring. Equally, the longer the generation time of the species the longer the timeframe needed for establishing 'success'. In order to demonstrate whether the 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.

8.4 Budget

There are many difficulties in deriving a generic budget for funding amphibian reintroductions. Because of the long-term nature of most reintroduction strategies it is probably unrealistic to persuade a single donor to commit funding for the entire duration of a project. However, a fund-raising strategy should be in place that should be consistent with the staged planning process mentioned above, so that breaks in the continuity of the project are avoided.

Recovery programs are often funded through short-term grants which often make maintaining continuity of expertise problematical. The coordinating body for a reintroduction program will usually be the local or national governmental conservation agency, and it will be the responsibility of this agency to ensure that the roles of different partners are clearly identified so that all parties are aware of their commitments. Personnel changes in either the lead agency or project partners can jeopardize reintroduction projects and the organization of the reintroduction program needs to account for this.

The logistics and costs of carrying out the activities required for a reintroduction program will vary by an order of magnitude between taxa and regions, and there are very few estimates of costs for any amphibian conservation programs. In England, the costs of carrying out development mitigation for great crested newt conservation—which embraces some but not all of the activities required for reintroduction—varied between UK£ 1350 and > UK£ 100,000 per project (Edgar *Et al.* 2005). This variation was largely due to differences in the scale of the projects undertaken—some lasted a few days while others extended to several years. The costs in Table 8.1 are based on reintroduction programs of four species of threatened amphibian in Europe (*Bufo calamita* and *Rana lessonae* in England; *Rana dalmatina* in Jersey; *Alytes muletensis* in Mallorca). The budget assumes that a thorough preliminary evaluation of the suitability of the species for reintroduction has already been performed by interrogation of the GAA, consultation with experts and literature survey. Some of the proposed activities may be short-term, and perhaps achieved within the timeframe of one year, while others will require a long-term commitment, but it is envisaged that no projects could be realistically completed in less than five years. However, the costs reflect the fact that certain activities (e.g. habitat management/threat mitigation) may require large initial outlays followed by rather lower annual maintenance budgets. Not all of the activities listed may be applicable to all projects and some projects may require specialist activities that are not listed. Economic circumstances may mean that projects carried out in tropical countries are proportionately cheaper, but this may be offset by higher travel costs and more difficult logistics. In most cases, reintroduction is likely to be a relatively expensive conservation option, particularly if it is combined with captive breeding. When a species can be conserved via habitat management/protection and/or threat mitigation the costs are likely to be considerably lower. Given current available expertise and methodologies, we propose that the ACAP reintroduction program should initially focus on a priority list of 20 species that will be compiled following the species selection process.

8.5 Acknowledgments

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Table 8.1. Suggested budget for carrying out an amphibian reintroduction program in Europe or North America. Costs shown are proposed costs (US $) per species for projects of up to 10 years duration (some projects may require > 10 years). Costs are based on travel, accommodation, equipment, consumables and overheads, but exclude staff salaries and/or student stipends.

	Duration of project		
Activity	1 year	5 years	10 years
Publicity, public relations and information	2000	4000	8000
Pre-release assessment of the wild populations	8000	40,000	/
Applied ecological research on life history and habitat requirements	10,000	50,000	/
Habitat management, restoration and creation and threat mitigation	15,000	35,000	45,0000
Population viability analysis and strategic recovery plan development	6000	/	/
Health monitoring and disease assessment	10,000	18,000	22,000
Genetic assessment	20,000	25,000	30,000
Local communities assessment	4000	/	/
Post-release monitoring	4000	20,000	40,000
Total	79,000	192,000	550,000

Chapter 9

The Continuing Need for Assessments: Making the Global Amphibian Assessment an Ongoing Process

S. Stuart

9.1 Introduction

The first phase of the Global Amphibian Assessment (GAA) was completed in October 2004, when the results were made public on the internet (http://www.globalamphibians.org/) and the headline findings were published in *Science* (Stuart *et al.* 2004). However, from its inception the GAA was always considered to be an ongoing program. The GAA is a joint project of the Species Survival Commission of IUCN—The World Conservation Union (IUCN/SSC), the Center for Applied Biodiversity Science at Conservation International (CI/CABS), and NatureServe.

9.2 Phase 1 of the GAA

The first phase of the GAA collected data on all amphibian species described by April 2004 (5743 species). The project was coordinated by a Central Coordinating Team of four people, three of whom (Simon Stuart, Janice Chanson, and Neil Cox) were based in Washington DC, with the fourth person, Bruce Young, in Monteverde, Costa Rica. The data were collected on a regional basis. The world was divided into 33 more or less arbitrary geographic units, and one coordinator (or occasionally more) was appointed. The GAA was being implemented in three stages, as follows: data collection; data review; data correction. The coordinators undertook the initial data collection, using an Access database provided by the Central Coordinating Team. These data were then reviewed by other scientists, usually in regional workshops, but also by other means. For this purpose, many of the GAA regions we combined; in total, 14 regional GAA workshops were held, plus a global workshop on caecilians. Following these review workshops, the Central Coordinating Team edited and corrected the data in the database, which invariably involved extensive follow-up communications with workshop participants. In total, about 520 scientists participated in the GAA.

As this process was completed, the Central Coordinating Team merged the different regional databases, merged species accounts for taxa that ranged across species boundaries, carried out a consistency check to ensure standardized approaches (especially in the application of the IUCN Red List Criteria), and undertook a basic global analysis of the data to produce the key findings of the GAA.

9.3 The need for continuous updating of the GAA

If the GAA is not kept up-to-date, its value will rapidly diminish. This is because the status of amphibians is changing rapidly, and scientific knowledge of amphibians is changing, possibly even more rapidly. Since the completion of Phase 1 of the GAA, over 140 new species of amphibian have been described. The number of known amphibian species will surpass 6,000 in 2006 (*Editors note:* this prediction was accurate).

For several reasons, it is important to keep the GAA up-to-date as a continuous process, rather than as an occasional "push every five years or so. This is because:

- Continual updating will keep the GAA database useful on an ongoing basis.
- Occasional updating will require clearing a massive backlog of changes and additions which would require a very large team of workers.
- Occasional updating is also likely to be more costly over the long-term, since it would require starting the project all over again, and rebuilding the relationships and networks of scientists on which the entire project depends.
- If the data are not kept current, it will be very hard to monitor the effectiveness of ACAP.

It is for the reasons that the GAA has always been considered to be an ongoing program.

9.4 The current status of the GAA

Since the completion of Phase 1 of the GAA, the Central Coordinating Team has continued the process of keeping the GAA up-to-date, and this has involved extensive communications with GAA participants (the number of whom has now increased to about 550 people). This updating has involved:

1. Numerous corrections of errors and omissions reported following the public release of the information.
2. Adding newly published information on range extensions, ecological requirements, threats and conservation status.
3. Adding newly described and revalidated species.
4. Reviewing and updating all the species accounts for the Mediterranean region at a workshop held in Spain in December 2004 (the workshop also covered all the reptiles of the region).

However, the ability of the Central Coordinating Team to keep on top of this work has been compromised by new demands on the time of all of the team members, in particular relating to the Global Mammal Assessment (Phase 1 is now 50% completed), the Global Reptile Assessment (started in five regions), and the Global Marine Species Assessment (just starting). In addition, team members are also working on two additional products that have been promised to all GAA participants: a CD of the database that will allow all participants to download the raw data and carry out their own research and analyses on it; and a book on the overall GAA results.

With these constraints on the Central Coordinating Team, it is clear that a new team now needs to be put in place, focusing on the GAA alone.

9.5 The Next Phase of the GAA

It is proposed that a new GAA Coordinating Team be recruited by IUCN to take over the running of the project, which should remain a collaboration between IUCN/SSC, CI/CABS, and NatureServe. This team should be devoted full time to the GAA, and should consist of a Program Director and a Data Manager. These people would interact on an ongoing basis with the growing scientific network of data providers. Their tasks would be:

- To add new species and revalidated species to the database, and to make all other changes to the data on the basis of new or previously overlooked information.
- To conduct three to four data review workshops each year, covering the entire globe every four years.
- To maintain and enhance the GAA web site, and to provide ways to make the raw data widely available, especially to the original data providers.
- To undertake analyses of the GAA data and to communicate and publicize important new findings.

The new GAA Coordinating Team should be in place during 2006. Priority regions for early data review workshops are:

- East Asia (China, and the Koreas): This region has one of the earliest workshops in Phase 1, and the data are now likely to be quite out of date, especially since the Central Coordinating Team does not have the capacity to monitor scientific papers in the Chinese language.
- Japan: There was no Japan workshop during Phase 1 of the GAA, and the data need to be augmented significantly.
- North America: Likewise, there was no workshop during Phase 1 of the GAA, and the distribution maps (which follow county boundaries in the United States) need to be improved.
- Mesoamerica: Several important experts from this region were not able to participate fully in Phase 1 of the GAA, and the data can almost certainly be enhanced through additional review.

Other important regions for workshops before the end of 2007 are: Brazil (where agreement on the Red List Categories for certain species still needs to be reached); Madagascar (where the distribution maps need to be made more accurate); India and surrounding countries (where major taxonomic changes are greatly altering the overall understanding of the amphibian fauna); and Australia (which was the first region to have a data review workshop in Phase 1 of the GAA). The plan is to complete a review of every region of the world by the end of 2009.

9.6 Budget

The approximate annual budget for maintaining the GAA continuous updating process is US $ 250,000 (for staff salaries and consultants), and US $ 120,000 for workshops, making a total annual expenditure of US $ 370,000. Although a significant cost, this is much less than the cost of occasional updating, and also much less than the anticipated conservation and research expenditures that will be required under ACAP. It is appropriate that such monitoring costs be a relatively small proportion of the overall ACAP budget, but, as outlined above, these expenditures are essential, because without the updating of the GAA it will not be possible to gain a comprehensive picture of the impact of ACAP in stemming amphibian declines and extinctions.

Chapter 10

Systematics and Conservation

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10.1 Introduction

Living amphibians comprise over 6000 known species, (AmphibiaWeb; Frost 2006) representing more than 20% of living tetrapods (Beebee and Griffiths 2005; Köhler *et al.* 2005). New species are being described at a rapid rate and in the last twenty years the number of recognized amphibians has increased, mostly by new discovery, by more than 40% (Köhler *et al.* 2005). With current levels of fieldwork we predict at least a 3% increase per year in the future.

The rate of discovery of new species exceeds that of any other vertebrate group; however, our knowledge of the amphibian fauna is still incomplete. The recent recognition of a large number of new species in areas such Sri Lanka (Manamendra-Arachchi and Pethiyagoda 2005), New Guinea (Tyler 1999), Madagascar (Glaw and Vences 2003), and the dozens of species described from the New World underscores the paucity of information we have even for large and diverse radiations. Amphibians inhabit most of earth's biomes. The diversity of species and life histories coupled with late twentieth century declines and disappearances worldwide make amphibians an important model for understanding the causes of global changes (i.e., climate change, earth warming, pollution, habitat loss, etc.) and their effect on biodiversity.

The total number of amphibian species is still unknown and as much as 50% of the amphibian fauna may still be undescribed. Furthermore, we know little about genetic structure, population size, and population dynamics in many parts of the world, particularly in tropical areas of the New World, Africa, and Asia. The recent IUCN assessment indicates that as many as a third of amphibian species are globally threatened (Stuart *et al.* 2004). However, comparing the proportion of threatened taxa to the total number of species is inevitably confounded by two problems: 1) the total number of amphibian species is uncertain, but certainly much higher than the current figure of just over 6000 described species (Collins and Halliday 2005); and 2) even among the currently described species, 22.5% are listed by IUCN as Data Deficient, and many of these could actually prove to be threatened (Stuart *et al.* 2004).

Worldwide tropical and subtropical forest amphibians require the most research emphasis to document amphibian diversity before it is lost. Many portions of the forested slopes of the Andes are at best inadequately sampled, yet we know that these forests hold one of the highest diversities of amphibians in the world, most of which have very small distributional ranges. Morphological and genetic diversification of Amazonian anurans are not correlated, morphological diversification underestimates the diversity of anurans throughout the area. The remaining Atlantic Forests and the mountains of Southern of Brazil, the Yungas of Peru and Bolivia, and forested areas in Paraguay are also known to have high amphibian species diversity, and most species have limited distributions.

10.2 Systematics: The Science Underlying the Establishment of Conservation Priorities

Systematics is generally regarded as the discipline of science focused on deciphering and understanding biological diversity. Systematics plays an important role in both biodiversity studies and conservation biology, from basic identification and classification of organisms to understanding population dynamics such as gene flow and population structure, to the final determination of areas of high diversity or endemism for prioritizing sites for protected status (Soltis and Gitzendanner 1998). The main areas in which systematics plays a unique role in setting conservation priorities are: a) taxonomic inventory (species identification and definition of species limits); b) identification of cryptic species; c) biodiversity and identification of hotspots; d) providing the phylogenetic framework to understand the history of biodiversity and the recognition of evolutionary significant units; and e) conservation genetics.

10.2.1 Taxonomic inventory: Species identification and definition of species limits

No names, no conservation. This simple statement encompasses both the most basic task of systematics and its central role in conservation initiatives. This basic and central role of systematics in conservation biology derives from the fact that any study (i.e., ecological, epidemiological, ecotoxicological, etc.) depends upon the accurate identification and classification of the species being studied. Consequently, resolving taxonomic uncertainties is basal to the assessment of biodiversity within and among areas. Taxonomic identifications based on morphological, ecological, molecular, and behavioral data provide the basis for assessing biodiversity.

It has been argued that areas where secondary contact between historically isolated lineages has been documented should be preserved because they can be regarded as natural laboratories for the study of evolutionary processes (Moritz 2002). Areas of intermixing between incompletely differentiated lineages might be common as a result of the nature of the speciation process, particularly in early stages of speciation. In the case of geographically restricted lineages this intermixing might lead to the extinction of the more restricted lineage. Comparative studies involving diverse taxa will help in identifying these areas, which are of special relevance for the preservation of unique lineages as well as evolutionary patterns and processes.

Focusing only on species counts can miss the important contribution of populations to ecosystem functions, particularly for species that are morphologically conservative but in which speciation occurs at the molecular level, resulting in a high level of genetic differentiation. Taxonomies based solely on overall similarities and differences in morphological characters undoubtedly hamper current conservation efforts by an underestimation of species richness in a given area.

An important limiting factor in our assessment of amphibian diversity is the pervasive lack of experts and trained individuals capable of describing species and identifying species in any given geographic region. This problem is accentuated if we consider that the large majority of trained systematists live in developed countries, whereas the vast richness of amphibian diversity is found in developing countries. In addition, the majority of developing countries have few trained individuals and lack resources to engage in training. Consequently, it is imperative that we undertake training in the form of in-country workshops that will train students and young professionals in all aspects of the systematic work from species identification, collection building and curation, analysis of characters, species descriptions, manuscript writing, and the scientific peer-review process. Workshops of this type are particularly needed in countries in Latin America, Africa, and Asia, where assessment of amphibian diversity is high (following the recent report of the Global Amphibian Assessment, GAA) and, at the same time, it is severely limited by a lack of properly trained individuals.

A large percentage of amphibian holotypes, i.e., the original described individuals by convention use as the reference representative of the species, and comparative collections are held at North American and European museums and universities; this also slows the work of colleagues in developing countries. The workshops described above need to be complemented with "systematic seed grants". These grants will support minor expenses for incidental work incurred in the process of describing species and will allow workers to visit museums and collections to have access to the needed comparative specimens. We anticipate that these grants will accelerate the "known but yet undescribed" species currently sitting in shelves and waiting description.

10.2.2 Cryptic species identification

The concept of cryptic species was introduced in the mid 20th century. However, only recently, with the advent of new techniques and methodologies, several studies have been especially effective in revealing large numbers of morphologically cryptic species within taxa that were previously recognized as comprising only a single species (Hanken and Wake 1998, 2001; Heyer *et al.* 2005b).

Because the taxonomic status of many amphibian species is still unknown, an apparently widespread and low-risk species may, in reality, comprise a complex of distinct taxa, some rare or endangered. Amphibians often manifest extreme levels of population substructure and genetic differentiation over relatively small geographic ranges (Hanken 1999). Cryptic species, which morphologically resemble one another so closely as to be indistinguishable, require special data for their resolution, such as advertisement call or genetic differentiation data (de Sá *et al.* 2004; 2005; Heyer *et al.* 2004; Heyer 2005). An understanding of the genetic diversity found in geographically widespread species has been successful in resolving those species as species complexes; that is, species encompassing more than one evolutionary lineage. The genetic differentiation within these species complexes exceeds the genetic diversity exhibited among other morphological distinct and well-supported species (Camargo *et al.* 2005). It is not unusal that for species that were originally identified as separate lineages in genetic studies subtle morphological, ecological, or behavioral differences are subsequently found (Heyer *et al.* 2005b; Heyer 2005).

Molecular phylogenetic studies of a diverse suite of threatened or endangered organisms have identified multiple genetically distinct lineages within units typically considered single species. These lineages are worthy of conservation; moreover, conservation and management strategies that do not consider such evolutionary units could prove harmful to the species (Soltis and Gitzendanner 1997).

10.2.3 Biodiversity and identification of hotspots

Understanding patterns of biodiversity may be the key to conserving remaining species, especially in tropical areas of the world. Biological diversity tends to be concentrated in "hot spots" corresponding to areas with historically high rates of geological change, rather than being uniformly distributed across a given habitat or zone. For example, tropical diversity is concentrated in South America, in the Indo-Malaysian region, and in the Eastern Arc Mountains of Africa, areas whose geological histories are extremely complicated.

Biological diversity has been shaped by millions of years of interactions between speciation and adaptation. Using the information available from phylogenetics and vicariance biogeography, we can identify those areas of endemism and high diversity that will ensure the preservation of evolutionary potential, make predictions about the characteristics of rare or poorly known species and begin to answer questions about the susceptibility of species and ecosystems to environmental perturbations.

Politicians and scientists now agree that a priority list of global centers for preservation of biological diversity is required. Diversity has generally been measured only in terms of species richness, or in the form of indices combining richness with abundance or, more recently, based on indices that contain cladistic classifications and give a measure of taxonomic distinctness. This measure of taxonomic diversity, when coupled with detailed knowledge of distribution, can be used in modified analyses of the type previously developed as 'critical faunas analysis' or 'network analysis'. For practical planning, two basic rounds of analyses are required: firstly, recognition of global priority areas by taxic diversity techniques; secondly, within any such area, analysis to identify a network of reserves that contains all local taxa and ecosystems.

The assessments of amphibian diversity require exploration of previously unvisited areas, comprehensive surveys of poorly known areas, and revisiting of localities that have not been assessed in the last decade. These biodiversity surveys undoubtedly will result in the discovery of many new species, but will also provide ecological, behavioral and genetic data to assess species complexes and levels of non-morphological differentiation. Tropical and poorly known areas should be a high priority of the biodiversity surveys. Furthermore, the GAA identified a large number of species as Data Deficient, meaning that the conservation status of their populations cannot be determined from the current available data. It is also important to assess the current population status and potential threats to Data Deficient species; it is likely that some of those species are already threatened with extinction. The activities outlined in a, b, and c, undoubtedly will collect and provide the information to fill in the gaps needed to determine the status of data deficient species and to establish the corresponding conservation priorities.

10.2.4 The phylogenetic framework and the recognition of unique lineages

Over the last century the assessment of amphibian evolutionary relationships has not kept pace with the description of amphibian diversity. Consequently, an evolutionary and phylogenetic framework is needed to evaluate amphibian declines. A phylogenetic framework of amphibian relationships, primarily derived from molecular data, has been recently presented (Frost *et al.* 2006) that should expedite this process. This framework is critical if we are to assess the causes of the declines or predict which species and lineages are at a higher risk of decline and extinction. Because of the large number of undescribed species and the existence of morphological conservative species complexes, it is difficult to determine the amount of genetic diversity that is being lost through reported declines and extinctions.

If the current amphibian declines have a historical component, i.e,. if they are phylogenetically constrained, then conservation efforts must center on the more susceptible clades. However, if a comprehensive phylogenetic analysis shows that the amphibian declines are randomly distributed among amphibian clades and lineages, then efforts need to focus on the causes of decline throughout the entire class.

Species represent history, and special attention should be given to the preservation of phylogenetic diversity. One way to approach this issue is to be attentive to maximizing total diversity across nodes on a phylogenetic tree (Faith 1992, 1994; Faith *et al.* 2004; Wall *et al.*, 1999), both globally and, to some degree, locally. In prioritizing taxa and habitats for conservation efforts, we seek to maximize both representation and persistence of diversity. The production of robust phylogenetic hypotheses for all species of amphibians is also the focus of the current AmphibiaTree project, (http://amphibiatree.org).

The efforts mentioned above will permit the recognition of narrowly endemic clades with very long branches (i.e., they are long-branch taxa with no close relatives). Identification of these clades and lineages merits special consideration in conservation efforts (Heyer *et al.* 2005a).

10.2.5 Conservation genetics

Genetic diversity is recognized as a fundamental component of biodiversity and its protection should be incorporated into conventions and policies to protect amphibians. Direct assessment of genetic diversity is often given low priority and the assumption made that protection of diversity at or above the species level will de facto protect the underlying genetic and evolutionary diversity. However this assumption is rendered suspect by the increasingly frequent detection of cryptic species (Moritz and Faith 1998; Camargo *et al.* 2005; Heyer *et al.* 2005).

Conservation genetics is the application of genetics to preserve species as dynamic entities capable of coping with environmental change. It encompasses genetic management of small populations, resolution of taxonomic uncertainties, defining management units within species, the use of molecular genetic analyses in forensics and understanding individual species biology. It deals with the genetic factors that affect extinction risk and genetic management regimes required to minimize these risks. Many amphibian populations have very small effective population sizes, commonly less than 100 (Funk *et al.* 1999). This makes amphibian populations especially susceptible to loss of genetic diversity by random drift, i.e., the random loss of alleles, and ultimately to the effects of inbreeding depression and high genetic load (Frankham *et al.* 2002). Conservation measures for declining amphibian populations will need to take account of this population structure, especially in already fragmented landscapes where the risks of population isolation are increasing with continuing fragmentation.

Both phylogeographic and phylogenetic patterns have roles in conservation biology at different levels, from evaluations of heterozygosity in threatened populations to analyses of population structure and intraspecific phylogeography to species-level issues and higher-level phylogeny. Analyses of genetic diversity in rare species occasionally identify one or more populations that are genetically distinct from other populations by virtue of either unique alleles or organellar genomes or allele frequencies. In some cases these populations or clusters of populations may warrant special management consideration because they represent unique genetic and, potentially, ecological components of species (Soltis and Gitzendanner 1997).

10.2.6 Safety box: Amphibian cell bank

Living animal cells can be cultured and frozen indefinitely for future conservation and research purposes, including cloning. A leader in this field of conservation technology has historically been the San Diego Zoo, with their establishment of a "Frozen Zoo" (http://conservationandscience.org/projects/gr_frozen_zoo.html) that holds biomaterials from approximately 7000 of threatened birds, mammals, and reptiles. Such a resource should not be considered as an alternate to any other conservation practice (e.g., habitat preservation), however if properly done (i.e., with well developed facilities and properly trained personnel to maintain them in the long run), but rather as a "life insurance policy" should other measures fail. It is in this light that it will be critical to expand this same concept of a frozen cell collection resource to include the world's threatened amphibian species. To guard against accidents and equipment failures, the frozen cells should be stored (duplicated) in two widely separated facilities. The San Diego Zoo's CRES (Conservation and Research for Endangered Species) could be one facility and the other could be established on another continent. The long-term goal of the Amphibian Cell Bank will be to store frozen cells from all of the world's threatened and endangered species of amphibians, estimated to be ~3000 species by the year 2010.

The samples of cells can come from many sources, including live amphibians collected along the biodiversity surveys described above that are needed to develop the phylogenetic framework, assess the genetic diversity, and to document the diversity of amphibians for conservation purposes. Because techniques of cell culturing vary with animal group, an initial phase will be required to develop the cell culture technology for amphibians and assess the feasibility of this project. (*Editors' Note:* Chapter 11 is devoted to the important issues, challenges and opportunities in the field of cryopreservation.)

10.3 Systematics as a Baseline for Conservation Studies

The phylogenetic framework of amphibian diversity will serve to analyze the effects of multiple other factors affecting our world today. Biodiversity surveys can identify how climate change is affecting and transforming the microhabitats by the changes in amphibian composition at any given site and by the decline of amphibian populations. Furthermore, the predictive power of phylogenetic hypotheses will suggest how other species (e.g., closely related species), may be affected by similar environmental changes. The same can be said about environmental contaminants and the effect that pollutant may have in amphibians, both in their aquatic as well as their terrestrial phase.

Solid evolutionary hypotheses for amphibians are also critical to understand both susceptibility and resistance to diseases, parasites, and pollutants. Given the current declines and the potential role of emerging infectious diseases in the declines, it is critical to understand the spread and reach of the diseases and other threats within a phylogenetic framework. Understanding the evolutionary history of amphibians is critical during the decision making process to select and determine which areas to protect. Clearly areas of high endemism, high diversity, and unique lineages will be identified in the resulting evolutionary hypotheses and will be critical in site planning. The distributional patterns resulting or enhanced by the biodiversity surveys will be the base line data for any attempt to make reintroductions.

10.3.1 Data needed to establish priorities

In summary, the critical raw data needed to delineate species limits and to formulate conservation planning for amphibians are: a) assign names to known undescribed species; b) inventories of poorly known areas; c) improved distributional data; d) assessment of genetic diversity; e) construction of phylogenetic frameworks to understand the history of amphibian diversity and its current decline

10.4 Five-Year Action Plan

The following activities are proposed:

- Naming species (1000 spp.).
- Training in-country students and auxiliary personnel (e.g, park guards, etc.) and support for in-country experts:
- Short term visitation of experts and students to research centers.
- Systematic workshops for students and young professionals.
- Grants to pursue graduate school or postdoctoral work in systematics, this grant could be for in-country or foreign institutions.
- Amphibians field surveys in poorly known areas and areas that have not being survey in the last decade.
- Genetic bar coding (1000 spp.).
- Frozen tissue bank of all taxa for molecular analysis and for cell banks.
- Evaluation of Critically Endangered and Endangered species in a phylogenetic analysis to prioritize taxa for conservation.
- Evaluation of Data Deficient taxa for conservation.
- Publication of field guides, in local languages.
- Establishment, improvement, and maintaining local collections.

10.5 Budget

The following table describes the cost of implementing different activities within the five-year action plan. It is recommended that the budget be dispersed on "seed" grants basis.

Cost	Description	Amount (US $)
Naming species	1000 species. Includes stipend for experts, funds for students/researchers to visit museum and collections abroad, stipend for technical assistants such as photographers and illustrators, supplies, software, DNA sequencing for two genes, publication costs.	10,000,000
Establishing genetic diversity and phylogenetic framework to detect cryptic species and understand declines	20 graduate theses per year.	5,000,000
Survey based on biogeographic patterns of diversity (GAA)	1. Neotropical Region 2. Sub-Saharan Tropical Africa 3. Asia/Pacific Region	5,000,000 3,500,000 3,500,000
Workshops and training	20 persons/workshop, 4 annual workshops per region (Latin America, Africa, Asia/Pacific, North America).	4,000,000
Web support (Digitizing Type specimens		500,000
Emergency supplies		250,000
Publications	20 Field Guides	400,000
Total		**32,150,000**

Chapter 11

Bioresource Banking Efforts in Support of Amphibian Conservation

O.A. Ryder

11.1 Introduction

Identifying successful strategies to confront declining amphibian biodiversity poses unprecedented challenges to conservation scientists. Much information about populations of amphibian taxa needs to be gathered, even as populations are disappearing at an alarming rate. Propagation in protected environments may prevent extirpation of populations and extinction of taxa, while simultaneously enabling the first studies of development for threatened and endangered amphibian species that are crucial to long-term persistence (Mendelson, *et al*, 2006).

The need to secure viable gene pools and self sustaining populations of endangered amphibians through insurance colonies in controlled environments will result in research studies into the genetics, physiology and reproduction of many amphibian species that were previously uninvestigated. As knowledge of amphibian fungal pathogens, especially *Batrachochytrium dendrobatidis,* advances, studies of the mechanisms of pathogenesis and of host resistance will assume a high priority in order to rescue amphibian populations, prevent pathogen transmission and begin to reestablish amphibian populations in regions from which they may have been extirpated (Mendelson, *et al.*, 2006).

One vital tool in recovery efforts is the establishment of biomaterials resources to support targeted research in support of reproduction, population management and disease prevention and treatment. Such efforts require close collaboration with efforts in animal collection, husbandry and pathology. To provide the most effective resource for current studies and offer the greatest variety of options for future managers, collection of tissues and germplasm, DNA and viable cell cultures represents an urgent need (Ryder, *et al*, 2000). Genome Resource Banks (GRBs) can provide materials that are essential for evaluating phylogenetic systematics, genetic variability, breeding biology and dispersal patterns, health and disease, as well as serving as a tool for enhancing reproduction and rescuing genetic variation that would otherwise be lost (Wildt, *et al.*, 1997; Ryder, 2002; Holt, *et al.*, 2004).

11.2 Opportunities and Challenges Involved in Bioresource Banking Efforts for Amphibians

Field teams now working to identify movement of the chytrid pathogen, *B. dendrobatidis*, through environments supporting amphibian populations and rescuing animals for *ex situ* propagation efforts may represent, in the extreme case, the last individuals who can collect samples for a diversity of biological studies from populations unperturbed by the sweep of this devastating disease. Collection of research samples most feasibly could come from salvaged specimens and entail no harm to wild or captive populations; rather, opportunistic sampling from individuals of varying life stages at post-mortem examination will be the most likely source of samples. The highest quality resource that might be obtained from such specimens would likely be viable cell cultures. However, only a sparse literature describing establishment and freezing of cell cultures from amphibians exists, and this from a very small number of taxa. Consequently, priority action in bioresource banking will need to concentrate effort on productive collaboration involving field biologists, captive breeding efforts, pathologists and those involved in cell culture and cell banking to assure the greatest possible number of taxa and sufficient numbers of individuals are sampled so that a global resource of frozen amphibian cell cultures is established.

The Global Amphibian Assessment (IUCN, 2006) recognizes in excess of 5,900 species; for most of these, genetic and reproductive information is limited or non-existent. Cell cultures are probably may only be available currently for a few species, such as African clawed toads, *Xenopus sp.* and the North American bullfrog, *Rana catesbeiana.* Genetic studies are numerous, but are largely focused on phylogenetic systematics. Significant studies of intraspecific genetic variation based, for example, on nuclear microsatellite allele analyses, have relevance for amphibian conservation efforts, but have been applied to relatively few species (e.g., Jehle and Arntzen, 2002). Perhaps, less than fifty amphibians currently have published microsatellite primer loci developed. Thus, in addition to rapid advances in amphibian husbandry and propagation needed to address needs identified in the Amphibian Conservation Action Plan, rapid progress in bioresource banking, especially cell culture methodology, in coordination with field collection of specimens for *ex situ* insurance populations is crucial.

11.3 Schema for Bioresource Banking

Rapidly, a network of individuals and institutions involved in collecting and maintaining rare and endangered amphibians, including animals removed from the wild as insurance populations, needs to be connected with laboratories experienced in establishing, maintaining and successfully freezing cell cultures. Such an unprecedented effort also needs to be conducted with appropriate curation and reference documentation, including quality assurance measures, such as vouchers, karyotypic and molecular characterization of cell cultures. With one institution leading in the effort to develop and improve the technology necessary for the challenges anticipated in attempting to culture cells from three diverse orders of amphibians, other, satellite, facilities will be established in countries rich in amphibian biodiversity and committed partnering institutions. Because of the successful history and broad accomplishments in mammalian, avian and reptilian cell culture, the advanced laboratory and information management infrastructure, and its location in the largest multi-disciplinary zoo-based research facility, the Frozen Zoo at the San Diego Zoo's center for Conservation and Research for Endangered Species (CRES) is suitable and willing to immediately undertake efforts to establish cell cultures for the first time as part of the Amphibian Conservation Action Plan.

11.3.1 Potential research and reproductive applications of amphibian cell cultures

Establishment of viable cell cultures can provide a renewable resource, making available high quality DNA, cellular RNAs, and cell fractions necessary for studies on chytrid fungal pathogenesis. In support of multi-disciplinary efforts to understand the basis of disease resistance and combat fungal disease, the development of *in vitro* model systems to facilitate studies of infection, replication and transmission will enable crucial studies. Studies of parasite-host interaction at the cellular level has formed the basis for development of therapeutic intervention in a wide variety of diseases and we may expect the same approaches to be utilized as the effort to protect small populations of amphibians in managed environments proceeds, provided, however, that resources for these studies are available. New proteomics platforms capable of characterizing and identifying proteins and the genes encoding them can be linked with the emerging information about the chytrid genomes as part of controlled studies utilizing cell cultures. In anticipation of such studies, efforts need to be immediately undertaken to establish cell cultures from diverse species of amphibians from uninfected populations and from populations that have survived chytridiomycosis.

Another potential application of viable cell cultures of amphibians involves the use of reproductive cloning to preserve genetic variation of populations that have been drastically reduced in size or to extinction. The production of amphibian embryos through same-species nuclear transfer into enucleated ova has been demonstrated (Briggs and King, 1952; Gurdon *et al.*, 1975) and, although much additional information must necessarily be obtained, including assessments of success utilizing heterologous ova (eggs not from the same species as the nuclear donor), the potential of cloning technology to assist in species conservation efforts cannot be ignored (Holt, *et al.*, 2004). Crucial to advances in assessing the practical difficulties and implementing application of amphibian cloning technology in the conservation context will be the availability of established cell cultures from a wide variety of amphibian species.

11.3.2 Technical considerations and methods for amphibian cell culture

Methods of cell culture and experimental utilization of cultured cells from amphibians is limited in comparison to other classes of vertebrates and insects. Cultured cells, e.g. fibroblasts, are routinely utilized for studies of mammalian chromosomes and skin biopsy specimens may be obtained at low risk to the animal being sampled. Most studies of amphibian chromosomes involve terminal experimentation and only a few established cell cultures are available from a limited number of taxa. Furthermore, many amphibian cell lines are derived from embryos or larvae. Nonetheless, it is possible to obtain cell cultures from adult specimens, including postmortem specimens. Clearly, much additional work needs to be done to develop methods to successfully establish cell cultures from samples that may become opportunistically available from various life stages of amphibians.

Assembling a diverse collection of amphibian cell cultures that can be utilized in studies of phylogeography, physiology, population genetics and fungal pathogenesis, including studies of factors leading to resistance, will require a broad participatory network of investigators linking field conservation efforts with *ex situ* programs for conserving amphibian gene pools and contributing to a better understanding of amphibian ecology and risk factors associated with amphibian declines.

11.4 Budget

There are two components to the Amphibian Cell Bank: (1) the tedious culturing of cells from each animal, prior to being frozen, and (2) the long-term storage of the cultured cells in liquid nitrogen. An estimated $500,000 is required to equip a lab for full cell banking capacity. An additional $500,000 represents an estimated cost for construction of a building that can house a fully functioning cell bank lab. In addition to such fully equipped hubs, a large number of satellite labs scattered around different countries provide several advantages. First, they allow for host country development of technology. Second, they permit storage of cells from species endemic to that country. Third, they potentially avoid the need to export live rare animals. A budget of $50,000 for each satellite facility would cover costs of the liquid nitrogen storage equipment (the bank part of the cell bank), and it would provide seed funds to develop the cell culturing capability. Additional funds would be necessary for long term maintenance. Even if cell culturing was not developed in a particular country, a satellite facility could still function by receiving frozen, cultured cells from another facility (hub or satellite) and exist as a bank only and important backup to other banks.

Cost	Description	Amount (US $)
Amphibian lab development at hub facility	Development of lab devoted to amphibian genome resource banking within a facility already designed to support such labs (ie. The Frozen Zoo at the San Diego Zoo's center for Conservation and Research for Endangered Species (CRES).	500,000
Hub facility and development of fully operational lab	Construction of a new hub facility and development of a lab capable of performing all steps in the cryobanking of species. This facility would serve as a backup to a primary hub (ie. CRES) and, together with the primary hub, help advance cell bank technology and support satellite facilities worldwide.	500,000
Sattellite facilities	Development of 50 satellite facilities with funding for banking species within a particular country or region and seed funds for equipping a lab with technology to culture cells.	2,500,000
Total		4,000,000

Chapter 12

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Appendix 1

Amphibian Conservation Summit

Washington DC, 17-19 September 2005

Declaration

Background

The amphibians – frogs, salamanders and caecilians – stem from an ancient lineage of organisms and they play essential roles, both as predators and prey, in the ecosystems of the world. Adult amphibians regulate populations of insects that are pests on crops, or which transmit diseases. The tadpoles of many amphibians, as herbivores or filter feeders, play a major role in aquatic ecosystems. Their well-being, or conversely their population declines and extinctions, signals that changes are occurring in the biosphere that have begun to negatively impact humans today.

Since 1970, scientists have observed precipitous population declines and outright disappearances of entire amphibian species. The extent of these declines and extinctions is without precedent among any other group of species over the last few millennia, and it has increasingly been the focus of scientific research. These declines have spread geographically and increasing numbers of species are involved. Recent research indicates that:

- Nearly one-third (32%) of the world's 5,743 amphibian species have been classified as threatened with extinction, representing 1,856 species.
- 122 species, perhaps many more, appear to have gone extinct since 1980. Further research may increase this number, since 23% of all species were classified as Data Deficient.
- At least 43% of all species have undergone population declines, but less than one percent is increasing in population size.
- As much as 50% of the amphibian fauna remains undescribed, and the possibility exists of discovering new groups that are widely divergent from any so far known.
- Habitat loss is the greatest threat to amphibians, impacting almost 90% of threatened species.
- A newly recognized fungal disease, chytridiomycosis, causes catastrophic mortality in amphibian populations, and subsequent extinctions.
- Many species are declining for reasons, such as disease, climate change, invasive species, and over-harvesting, that cannot be readily addressed through traditional conservation strategies.
- Other issues, such as the role of environmental pollutants in amphibian declines, need to be more thoroughly addressed.

Since 1990, scientists have referred to amphibians as canaries in the coal mine; the Global Amphibian Assessment (GAA) shows that the canaries are dying. This underscores a weakness in current strategies for biodiversity conservation: that habitat conservation is essential but not sufficient. Existing protected areas alone are not sufficient to protect amphibians from a growing array of threats.

The Amphibian Conservation Summit was called because it is morally irresponsible to document amphibian declines and extinctions without also designing and promoting a response to this global crisis. To this end, the Amphibian Conservation Summit has designed the Amphibian Conservation Action Plan (ACAP), and commends it to governments, the business sector, civil society and the scientific community for urgent and immediate adoption and implementation.

Amphibian Conservation Action Plan (ACAP)

Four kinds of intervention are needed to conserve amphibians, all of which need to be started immediately:

1. Expanded understanding of the causes of declines and extinctions
2. Ongoing documentation of amphibian diversity, and how it is changing
3. Development and implementation of long-term conservation programmes
4. Emergency responses to immediate crises

1. Expanded understanding of the causes of declines and extinctions

A. Emerging amphibian diseases. Emerging diseases are a major threat to the survival of human populations globally. Diseases like SARS, HIV/AIDS, Ebola, and avian influenza emerge because of changes to the environment (e.g., encroachment into wildlife habitat) and human behaviour (e.g., trade and travel). At the same time, a series of wildlife diseases have emerged, threatening many species. These are products of the same underlying causes – anthropogenic environmental changes – and highlight the growing link between conservation of biodiversity and the protection of human health.

Of the diseases known from amphibians, one, chytridiomycosis, is clearly linked to population declines and extinctions. This fungal disease is appearing in new regions, causing rapid population disappearances in many amphibian species. It is the worst infectious disease ever recorded among vertebrates in terms of the number of species impacted, and its propensity to drive them to extinction.

A series of strategies to deal with disease in the field is needed. Research should focus on understanding why some species of amphibians become extinct in some regions and at certain times, while others do not. This will require studying the persistence of the pathogen, reservoir hosts, mechanisms of spread, interactions with climate change, and comparing disease dynamics between sites of declines and control sites where amphibians survive. Research is also urgently needed on the biology of this emerging pathogen, in particular on:

- how it causes death;
- how amphibians respond by developing immunity or changing behaviour;
- understanding the geographic distribution and dispersal of chytridiomycosis; and
- whether or not animals from decline and control sites differ in their responses to chytridiomycosis.

These research programmes should also consider possible interactions between disease and other factors involved in amphibian declines (such as climate change, habitat loss or contaminants) and mechanisms for dealing with them (such as captive breeding and reintroduction).

To implement this research on disease, *Regional Centers for Disease Diagnostics* will be set up in Latin America, North America, Europe, Australia, Asia and Africa. They will provide free testing to field research groups, and will manage the logistics for regionally based *Rapid Response Teams*. A seed funding system should be created to support imaginative approaches to stopping outbreaks from spreading and preventing extinction by infection.

B. Climate change. Evidence of a link between amphibian declines and climate change is growing. Changes in temperature or precipitation influence host-pathogen interactions, and short-term and seasonal patterns in amphibian behaviour. One consequence is an increase in the probability of outbreaks of lethal diseases such as chytridiomycosis. If efforts to address climate change remain inadequate, none of the other proposed conservation efforts can save amphibians in the long term. The current spate of extinctions might be the first wave in a more general, profound loss of biodiversity. Ultimately, preventing this requires greater political will to take all necessary measures to reduce human impact on the global climate.

Research is needed to understand how climate change affects amphibians, and why the impacts are greater today than they were historically. In particular, studies should focus on the impacts of climate change on disease dynamics, and should develop predictive models for future declines, thus enabling implementation of conservation measures. Research will also explore ways in which ecosystems could be made more resilient to climate change (such as measures to restore movement corridors that would ensure metapopulation functions or allow migration to new habitats), and whether or not there might be ways to manipulate local micro-scale climates.

C. Environmental contamination. Contaminants may have strong impacts on amphibian populations by negatively affecting immune function and causing infertility, developmental malformations, feminization, endocrine disruption, and alterations in food webs. There is evidence that environmental contaminants can cause local amphibian declines and extinctions. The effects of contaminants on broader geographic scales such as watersheds are not well understood. An ecotoxicology consortium should be formed in order to determine: how contaminant loads differ between stable and declining populations; the relationship between declines and contaminants in all regions; the effects of major chemical classes on both the aquatic and terrestrial life stages of amphibians; the effects of sub-lethal exposure in the presence of other threats such as disease; the role of contamination in amphibian population declines at the landscape scale; whether or not present regulatory screening is adequate; approaches to minimize the movement of chemicals through the environment; and how well the future impacts of contaminants can be predicted. The research should be integrated with the work of the regional centers recommended for disease research and management.

2. Ongoing documentation of amphibian diversity and how it is changing

A. Exploration and biodiversity evaluation. Without an understanding of the amphibian fauna, its history, and its distributional patterns, conservation priorities cannot be set rationally. Therefore it is essential that basic exploration and species descriptions continue. The rate of species description among amphibians is higher than it has ever been. However, in many parts of the world, especially in the tropics, knowledge of amphibian species, their distributions, and their requirements for survival is still too poor to enable reliable conservation priorities to be identified. The ACAP will implement a greatly enhanced programme to: name at least 1,000 new species in five years, and 2,500 species in ten years; understand species limits and resolve species complexes; and carry out inventories of amphibian faunas. The implementation of this programme will require major building of taxonomic capabilities in a number of tropical countries, with priority being given to poorly known areas, and areas of high endemism and diversity. To assist in identification of species, new field guides and internet resources should be produced. Innovative mechanisms should be developed to enable taxonomists to devote more time to high priority work. Research should also focus on: Data Deficient species; identification of unique and ancient evolutionary lineages; understanding the extent to which similarity in vulnerability to threats is determined by degree of relatedness between species; and whether genetic diversity of species relates to their ability to persist in the face of an array of threats.

B. Updating the Global Amphibian Assessment continuously. An accelerated programme of assessment must underpin the ACAP. To build on its initial success, the GAA needs to be maintained continuously by: establishing a new full-time GAA coordinating team; recording updates and corrections to the data; developing more efficient mechanisms within regions to update the data; making the data more widely available; maintaining and enhancing the GAA web site; and undertaking analyses and communicating findings. A complete update of the GAA should be finished by 2009. Particular emphasis should be given to improving discrimination between real and apparent declines.

3. Development and implementation of long-term conservation programmes

A. Protection of key sites for amphibian survival. Habitat loss and degradation are impacting nearly 90% of threatened amphibians. Most of these require habitat- or site-based conservation as the primary means to ensure their survival. Therefore, safeguarding key sites for threatened amphibians is the most urgent priority for the survival of many species. At least 940 amphibian species (422 of which are threatened with extinction) are not in any protected areas. An urgent priority of the ACAP is to identify the highest priority sites, using globally recognized, standardised, and quantified criteria, which are essential for the survival of threatened species that are currently receiving no effective conservation measures. These sites and their associated landscapes need urgent attention, such as protected area establishment, community level sustainable development, and local education and training. The ACAP will establish a site conservation programme with the following main elements: identifying the 120 highest priority sites; and applying appropriate conservation actions at each site, including the development and implementation of management plans, standardised monitoring and assessment protocols, and long-term sustainability plans for ongoing funding and management. Given that what goes on outside a key amphibian site will hugely impact the success of conserving that site, management plans should incorporate the need to protect ecosystem services at a broad ecological scale. This site conservation programme will involve governments, non-governmental organizations, community-based organizations and the business sector collaborating to bring about effective conservation in the highest priority sites, with the widest possible stakeholder support.

B. Reintroductions. The goal of reintroduction is to re-establish protected, viable amphibian populations in the wild where conventional habitat management and threat abatement alone are unlikely to result in population recovery. Many amphibian reintroductions will be needed once techniques for the management of chytridiomycosis and other threats become available. Experience and expertise in amphibian reintroductions need to be developed as a matter of urgency. The ACAP will determine which species will benefit from reintroduction programmes by developing and applying rigorous and objective criteria. Once the species have been selected, reintroduction programmes will be initiated. The animals used for reintroductions may either stem from captive breeding programmes or wild populations, depending on availability of stock and the nature of the circumstances. In the first instance, it is estimated that 20 species will be selected for reintroduction, but this may increase as funds and capacity are built.

C. Control of harvesting. In some parts of the world, especially in East and Southeast Asia, but also in some other tropical countries, unsustainable harvesting of amphibians, especially for food and medicines, has led to severe population declines. There are also instances of declines due to the international pet trade. The ACAP will establish a harvest management programme, concentrating on 15 countries that appear to be the focus of the heaviest levels of harvest. The programme will build management capacity in each of these countries to halt declines due to over-harvesting, with an emphasis on: the development of sustainable use projects (when the biology of the species permits this); the development and strict enforcement of appropriate legislation; monitoring the levels of amphibian harvests and trade; the implementation of recovery plans for the most threatened species; the certification and regulation of commercial captive breeding operations with a proportion of profits returning to conservation in the wild; and raising awareness of the impacts of unsustainable use of amphibians. Commercial captive breeding facilities should only use species native to their regions to reduce the risk of the spread of disease and alien frogs. Species that are threatened by international trade should be listed on the appropriate appendices of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES).

4. Emergency responses to immediate crises

A. Rapid response capacity. The short timescale of many amphibian declines requires the capacity for immediate response, as already mentioned. The regionally based *Rapid Response Teams* required to react to disease outbreaks should be established and implemented urgently.

B. Captive survival assurance programmes. The ACAP recommends prioritized (as outlined below) captive survival assurance programmes that are primarily in-country, coupled with an obligation to deliver *in situ* threat mitigation and conservation programs. This is both a stopgap to buy time for species that would otherwise become extinct, and an integral component of other approaches to tackling amphibian declines. Guidelines for including species in captive survival assurance programmes will be based on predictive models of threats so that species are targeted proactively and representative populations are collected. Decision processes will involve consultation with representatives across the ACAP consortium and the range country will be the ultimate arbiter.

Several hundred amphibian species, perhaps more, are facing threats such as disease and climate change that cannot be addressed in the wild with currently available conservation management strategies. Since solutions for the conservation of these species in the wild are not currently available, a short-term solution is to breed them in captive survival assurance colonies to maintain options for reintroduction. Capacity to implement a major captive programme for amphibians does not currently exist anywhere in the world. Therefore this should be achieved through the establishment of an Amphibian Survival Alliance to coordinate this effort globally, involving rapid-response teams to collect disappearing species, short- and long-term captive management, training and capacity building for captive conservation programs in range countries, research on captive breeding and reproductive science, disease management, and education and outreach. Captive programs will include a variety of operations from rapid-response, portable units, to large-scale permanent facilities. The goal is to maintain and breed in captivity species at risk of extinction, which should be collected from places where declines have not yet occurred, as well as from places where animals need to be rescued urgently before they disappear.

C. Saving sites about to be lost. The integrity of some of the top priority sites for amphibian survival is under immediate threat. In some cases, habitats are reduced to tiny fragments that will disappear very soon. An "amphibian emergency fund" should be established to implement immediate conservation measures in such sites before it is too late.

D. Saving harvested species about to disappear. Several species are close to extinction due to over-exploitation. The "amphibian emergency fund" should be used to address threats to these species.

Amphibian Action Fund

The implementation of the ACAP over the period 2006-2010 will cost approximately US$ 400 million. To help support the implementation of the ACAP, the Amphibian Conservation Summit announced the formation of the Amphibian Action Fund and received initial pledges from donors.

The Amphibian Action Fund will support:

1. Expanded understanding of the causes of declines and extinctions
2. Ongoing documentation of amphibian diversity, and how it is changing
3. Development and implementation of long-term conservation programmes
4. Emergency responses to immediate crises

Supporting a network of amphibian experts

The ACAP cannot be implemented without a global network of scientists and conservationists who work on amphibians. To date, the IUCN Species Survival Commission (IUCN/SSC) has focused on decline-related research through the Declining Amphibian Populations Task Force (DAPTF), on promoting conservation through the Global Amphibian Specialist Group (GASG), and on monitoring and assessments through an informal network of scientists contributing data to the GAA. All three of these programmes have made significant achievements, but all of them are also struggling for resources, and are based on broadly the same network of experts. In view of the extraordinary nature of the crisis facing amphibians, the IUCN/SSC should bring these three programmes together in a single Amphibian Specialist Group (ASG) focused on conservation, research and assessment. The ASG needs to have sufficient resources and finances to lead the implementation of the ACAP.

Conclusion

The Amphibian Conservation Action Plan is the most ambitious programme ever developed to combat the extinction of species. This response is necessary because the amphibian extinction crisis is unlike anything that the modern world has previously experienced, and a large proportion of amphibian diversity remains undocumented. The ACAP requires the international community to enter uncharted territory and to take great risks. But the risks of inaction are even greater. The Amphibian Conservation Summit calls on all governments, corporations, civil society and the scientific community to respond to this unprecedented crisis. There needs to be unprecedented commitment to implementing the Amphibian Conservation Action Plan with accompanying changes in international and local environmental policies that affect this class of vertebrate animals. They are indeed canaries in the global coalmine.

Appendix 2

Amphibian Conservation Summit

Washington, DC, 17–19 September 2005

Delegation

Ross Alford, James Cook University, Australia
Franco Andreone, Museo Regionale di Scienze Naturali de Torino, Italy
Ariadne Angulo, Conservation International, Andes CBC
Michelle Boone, Miami University, USA
Kurt Buhlmann, University of Georgia, USA
Kevin R. Buley, Chester Zoo, UK
Rafe Brown, University of Kansas, USA
Cynthia Carey, University of Colorado, USA
Ana Carolina O. Q. Carnaval, University of California, Berkeley, USA
Gonzalo Castro, Global Environment Facility, USA
Don R. Church, Conservation International, USA
James P. Collins, Arizona State University, USA
Luis A. Coloma, Universidad Católica del Ecuador, Ecuador
Steve Corn, United States Geological Survey, USA
Deborah Cowman, United States Geological Survey, USA
Andrew Cunningham, Zoological Society of London, UK
Peter Daszak, The Consortium for Conservation Medicine, USA
Carlos Davidson, San Francisco State University, USA
Andrew P. Dobson, Princeton University, USA
Holly Dublin, IUCN Species Survival Commission, Switzerland
Xie Feng, Chinese Academy of Science, China
Chris Funk, University of Texas, Austin, USA
Michelle Gadd, United States Fish and Wildlife Service, USA
Ronald Gagliardo, Atlanta Botanical Garden, USA
Gerardo Garcia, Durrell Wildlife Conservation Trust, UK
Claude Gascon, Conservation International, USA
Richard Gibson, Zoological Society of London, UK
Richard Griffiths, The Durrell Institute of Conservation & Ecology, UK
Scott Hajost, World Conservation Union, USA
Tim Halliday, Open University, UK
James Hanken, Harvard University, USA
Reid Harris, James Madison University, USA
Tyrone Hayes, University of California, Berkeley, USA
Blair Hedges Pennsylvania State University,USA
Ron Heyer, Smithsonian Institution, USA
Bill Hopkins, Virginia Polytechnic Institute and State University, USA
Les Kauffman, Boston University, USA
Rick Kearney, United States Geological Survey, USA
Sergius Kuzmin, Russian Academy of Sciences, Russia
Robert Lacy, IUCN/SSC Conservation Breeding Specialist Group, USA
Sally Lahm, no affiliation
Esteban Lavilla, Fundacion Miguel Lillo, Argentina
Karen R. Lips, Southern Illinois University, USA
Michael Wai Neng Lau, Kadoorie Farm and Botanic Garden, Hong Kong, China
Michael Lannoo, Indiana University School of Medicine, USA
Thomas Lovejoy, H. John Heinz III Center for Science, Economics, and Environment, USA
Stefan Lötters, University of Amsterdam, Netherlands
Dan Martin, Conservation International, USA
Jeanne McKay, The Open University, UK
Joseph R. Mendelson III, Zoo Atlanta, USA
George Meyer, Los Angeles, California, USA
Scott Miller, National Zoological Park, USA
Joseph Mitchell, University of Richmond, USA
Russell Mittermeier, Conservation International, USA
James B. Murphy, United States National Zoological Park, USA
Mike Parr, American Bird Conservancy, USA
Gabriela Parra, Universidad Autonoma de México, México,
Rohan Pethiyagoda, The Wildlife Heritage Trust of Sri Lanka, Sri Lanka
Bruno Pimenta, Museu Nacional do Brasil, Brasil
Budhan Pukazhenthi, United States National Zoological Park, USA
J. Alan Pounds, Monteverde Cloud Forest Reserve, Costa Rica
George Rabb, Chicago Zoological Society, USA
Jean Raffaelli, Urodela Specialist Group, France
Rick Relyea, University of Pittsburgh, USA
Stephen Richards, James Cook University, Australia
Rob Riordan, NatureServe, USA
Santiago Ron, University of Texas, Austin, USA
Mark-Oliver Rödel, University of Würzburg, Germany
Andrew Sabin, New York, USA
Rafael O. de Sá, University of Richmond, USA
Luis Schiesari, University of São Paulo, Brasil
Débora Silvano, Secretaria de Biodiversidade e Florestas, Ministério do Meio Ambiente, Brasil
Bruce Stein, NatureServe, USA
Simon N. Stuart, IUCN Species Survival Commission; Conservation International, USA
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Tracey Tuberville, University of Georgia, USA
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