

Honduran Amphibian Rescue and Conservation Center

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Executive Summary

The endangered amphibians of Honduras are experiencing a storm of assaults from habitat destruction, climate change, and emerging infectious diseases. A growing number of species face an uncertain future unless ex situ management efforts are soon implemented to ensure long-term survival. From 2006 to present, I have been studying the amphibian populations of Cusuco National Park (CNP), a cloud forest recognized by the Alliance for Zero Extinction for the critical habitat it provides for endemic Honduran amphibians. There are seven amphibian species which can be only be found in CNP, a forest fragment about 10 mi x 12 mi in size. Amphibian abundance surveys conducted from 2007 to 2011 show an overall decline in the presence of stream-associated amphibians in CNP (Fig. 1).

Amphibian chytrid fungus (Bd) has proven to be especially devastating to amphibian populations in Latin America, but the amphibians of Honduras have received little conservation attention relative to countries where Bd is believed to be a recent introduction. Although there is a common perception that extinction-prone species will primarily succumb rapidly only upon initial exposure, I recently completed a 5-year Bd infection survey in Honduras that demonstrates otherwise; Bd-associated amphibian declines are still occurring even after long-term pathogen presence (Fig. 2). To address this concern, the current project will ensure long-term survival of three Critically Endangered species in CNP: *Plectrohyla dasypus*, *Plectrohyla exquisita*, and *Duellmanohyla soralia* (Fig. 3). Illegal deforestation and chytridiomycosis are negatively impacting each life stage of these species, culminating in the shrinkage of adult populations and pushing them deeper into an extinction-prone bottleneck. The rescue efforts proposed herein will tackle both short-term and long-term threats by performing a head-start program to quickly produce animals for wild populations supplementation while simultaneously building captive assurance populations for long-term protection and reintroduction.

Introduction

All three of my target species (*Plectrohyla dasypus*, *Plectrohyla exquisita*, and *Duellmanohyla soralia*) are currently threatened with extinction by the presence of amphibian chytrid fungus, which is now exacerbated by increasing habitat degradation in and around CNP. Before I began my study in 2006, mysterious amphibian declines had already been reported in CNP despite habitat protection, as described in the IUCN Red List assessments which served as the basis for my Bd investigations. I have since performed five years of Bd research in CNP and discovered alarmingly high rates of infection in these critically endangered species and erratic fluctuations in the infection rates between successive years.

Detecting high infection rates does not necessarily mean that a species is declining from chytridiomycosis, which is why I have also been conducting simultaneous species abundance surveys and mark-recapture surveys for the past 5 years. My data now provides a clear association between the disappearance of amphibians with those species that have demonstrated a high susceptibility to Bd infection (stream-associated species). Furthermore, a 4-year mark-recapture survey of *D. soralia* shows a direct link between an increase in Bd prevalence, a rise in annual precipitation and simultaneous population decline during that year (Fig. 2). Similar surveys are being conducted for *P. dasypus* and *P. exquisita*, but adults are already much less abundant and recapture rates have been too low to produce reliable population estimates. These findings are alarming, and suggest that the future of these species is linked to the prevalence of Bd and that this is in turn affected by unpredictable and uncontrollable abiotic factors such as mean annual rainfall rates. This is worrying because it has previously been assumed that amphibian populations in Honduras should have reached stability from the effects of a historic Bd introduction (15+ years ago). My work shows that there are still critically endangered amphibians in Honduras at risk of extinction from Bd, especially when coupled with encroaching habitat degradation that may soon catalyze increased declines resulting from stress-induced immunosuppression and a rise in chytridiomycosis. In a parallel project, Operation Wallacea is currently working to protect CNP from illegal deforestation. Accordingly, my project will also help raise the profile of this park's conservation significance and the need to protect it, as CNP is home to 10 critically endangered, 6 endangered, and 7 near threatened amphibian species.

Methodology

My previous research suggests that the life stage most highly impacted by Bd in CNP are recently metamorphosed frogs. I have sampled all life stages of each species simultaneously to produce a life history cross-section and each year, consistently detect both the highest prevalence and intensity of infection at this stage when the amphibian's immune system is most vulnerable to Bd. It appears that most of these metamorphs do not survive and that this is causing the decline in adult populations observed. Very few adult frogs are testing positive for infection, and those that do have only very mild infection intensities as per qPCR. Therefore, the foundation of this proposed rescue project is to supplement wild adult populations, initially through head-starting, whereby tadpoles will be collected from the wild, treated for Bd infection in captivity, and raised in a biosecure amphibian rescue center within Honduras to prepare them for reintroduction to CNP as healthy subadults (>50% adult SVL). Since there is currently debate over the best treatment method and life stage at which to administer treatment (as per my communications with Allan Pessier), we will conduct experimental trials early in this program to determine the most effective and safe method for our target species (tadpole vs metamorph, itraconazole vs chloramphenicol, etc.). All animals will be screened for Bd via PCR upon arrival in quarantine and periodically be reevaluated prior to release. PCR samples will initially be processed at Washington State University until I build local capacity at the national university in Honduras to run Bd PCR and process our samples over the long-term. The reason I believe reintroductions will be successful is because the adults of all 3 species are consistently testing negative for Bd, or show only very low level infections compared to earlier life stages, hence they do not appear to be threatened by Bd like the earlier life stages. My long-term goal is to establish self-sustaining captive assurance populations to ensure the survival of these species.

Three biosecure amphibian rooms will be constructed to house this project within a building earmarked by the Honduran Government at the research center of Cuyamel Omoa National Park,

located approximately 10 miles northwest from CNP (the necessary infrastructure does not exist in CNP). These rooms will be constructed by myself and Jessi Krebs along with local help, measuring approximately 8'x8'x8' each and modeled after those already successfully constructed and maintained by Krebs at the Henry Doorly Zoo (Fig. 4). One of these rooms will serve as a quarantine facility, where all new animals will be housed until treated and confirmed Bd-negative and the other two will contain animals being raised and prepared for reintroduction. These isolated amphibian rooms will employ high standards of biosecurity, be completely watertight, and locked to prevent unauthorized access. Amphibian keepers will wear only dedicated footwear and clothing when entering these rooms, and all water both entering and exiting these rooms will be treated to prevent introduction of Bd (by using RO water) and also to prevent the release of contaminated water (by treating with bleach for 12+ hours before disposal). Fresh examination gloves will be worn at all times and no materials will be shared between the rooms nor will any animals enter these rooms that were not collected from CNP. We will experiment with different insect cultures of species found in Cusuco to create a stable captive food source without the risk of introducing disease agents from continually wild-caught foods. For the duration of this long-term project, annual population surveys will be conducted in CNP to monitor wild populations and evaluate the success of reintroduction efforts. All reintroduced animals will be marked using Visual Implant Elastomers to provide a unique fingerprint for subsequent mark-recapture surveys. The use of PIT tags will also be considered.

To execute this project, I have developed a multinational partnership between Honduras (with ICF, the Government's Wildlife Department), the USA (the Henry Doorly Zoo), and the UK (Operation Wallacea). I have discussed this project with the Honduran Government (ICF), who has expressed full support and has agreed to grant all necessary permits (see attached letter). The goal of this project is not only biodiversity conservation, but also local capacity-building, which I believe is imperative for successful long-term conservation projects within range countries. The facility will be run by local Honduran biologists, with my full support and supervision in the background. I am often present in Honduras for 2 months/year and am committed to making myself available to supervise the facility for additional time upon start-up. Training in amphibian husbandry for staff will be provided by Jessi Krebs at the Henry Doorly Zoo, who will host their visit to the Zoo and provide opportunities for hand-on experience working in their isolated amphibian rooms. Additional training opportunities for staff will be pursued including attendance at the Amphibian Biology, Conservation & Management class provided by AZA. I will also work to establish a student internship program for Honduran university biology students to volunteer with the project and gain amphibian husbandry and field survey experience. My short-term goal is to construct the amphibian rescue facility and train local staff in preparation for the first field collection of wild amphibians in 2013. Funding to continue this project will be sought from the Columbus Zoo and Aquarium and from Rufford Small Grants, both of whom have already funded earlier field research components of this project.

Current Budget

| Budget Category | Item/Amount | Requested from AArk | Other sources/status |
|-------------------------|--|---------------------|--------------------------------------|
| Field Research | RT flight to Honduras x 1 | 0 | \$600 provided by Operation Wallacea |
| | Visual Implant Elastomers (to establish a new long-term population survey) | 0 | \$500 provided by Operation Wallacea |
| | Vantage Vue Weather Stations and data loggers (collect addit'l environmental husbandry data) | 0 | \$820 awarded in 2010 |
| | Chytrid infection survey | 0 | \$5000 Requested from MBZ |
| Ex situ Facility | Construction materials for Biosecure Amphibian Rooms | \$3000 | \$5000 Requested from MBZ |
| | Electrical & Plumbing upgrade on current building | 0 | \$2000 Requested from MBZ |
| | Industrial Rack Systems x 6 (4/room x 3) | 400 | \$1000 Requested from MBZ |
| | 15-gallon aquariums x 80 (20/species x 3; + 20 for quarantine) | \$800 | \$2000 Requested from MBZ |
| | Air conditioning unit x 3 | \$400 | \$1200 Requested from MBZ |
| | PVC pipes and plumbing for drainage systems | 0 | \$1350 Requested from MBZ |
| | Compact florescent light fixtures & bulbs (6/ room x3) | 0 | \$3780 Requested from MBZ |
| | Disposable gloves x 1,000 prs | \$200 | 0 |
| | Live food culture equipment | \$200 | 0 |
| Total | | \$5,000 | \$23,250 |
| Percentage | | 17.7% | 82.3% |

Timeline of Work

| Activity | July-Sept 2012 | Oct-Dec 2012 | Jan-March 2013 | Apr-June 2013 |
|---|----------------|--------------|----------------|---------------|
| Fieldwork- Continue Pop Surveys and Environmental Monitoring | X | | | |
| Construct ex-situ Facility | X | X | | |
| Training Staff/Education | | X | X | |
| Field Collection of Amphibians | | | | X |

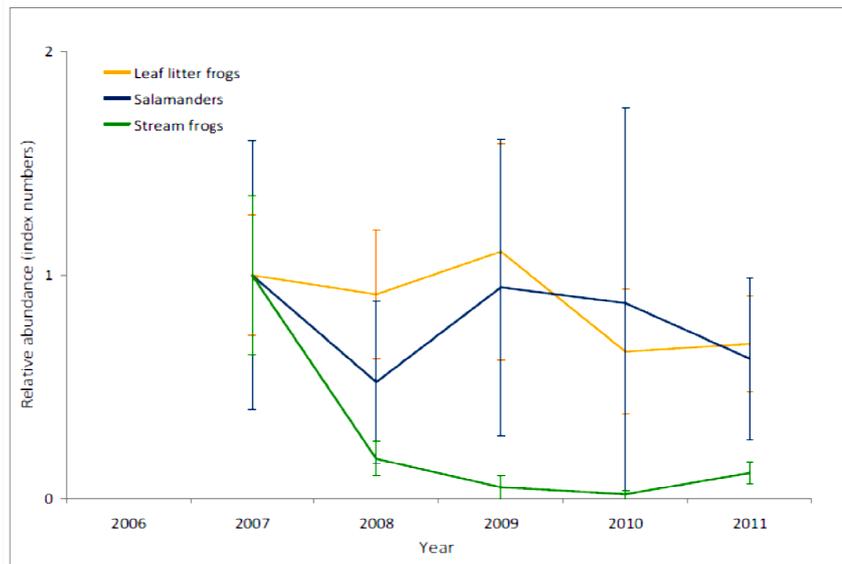


Figure 1. Amphibian encounter rates in Cusuco National Park, 2007-2011. These data provide evidence for a marked decline in stream-associated amphibians over the past 5 years, which largely consists of the three species targeted by the proposed rescue efforts. Figure extracted from the Operation Wallacea 2011 Status Report: Cusuco National Park available at: <http://www.opwall.com/Library/Research%20Reports%20-%20Honduras%20Terrestrial.shtml>

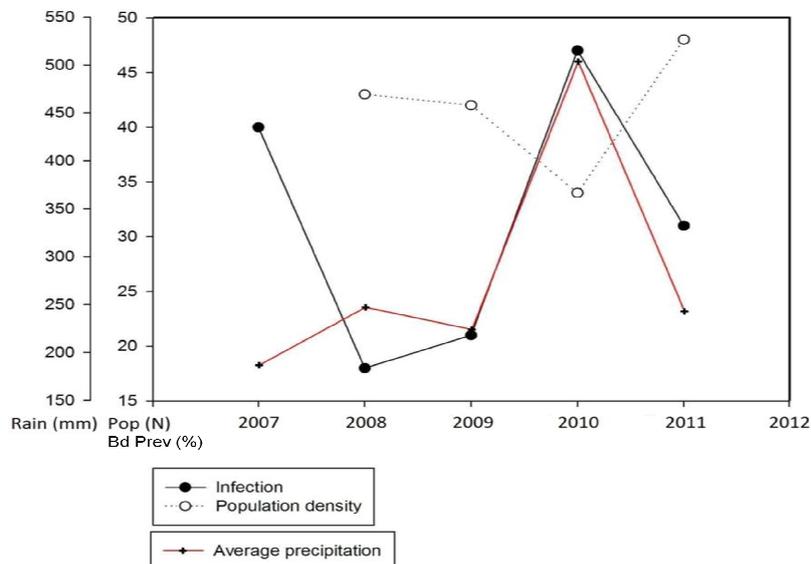


Figure 2. Four-year population trend of adult *Duellmanohyla soralia*, chytrid infection prevalence, and relative annual rainfall in Cusuco National Park, Honduras, illustrating the negative effect of Bd infection on the number of adult amphibians. The close positive association between Bd prevalence and average annual precipitation further suggests that several consecutive years of high rainfall may be enough to catalyze a significant Bd-related population decline in *D. soralia*, the only species still abundant enough to produce reliable mark-recapture data. Now is the time to begin augmenting populations before this occurs.

Figure 3. Critically endangered species to receive ex situ management through this project:

Plectrohyla dasypus: Critically Endangered, Endemic to CNP



Plectrohyla exquisita: Critically Endangered, Endemic to CNP



Duellmanohyla soralia: Critically Endangered, Highly Fragmented & Restricted Distribution



Figure 4. Biosecure Amphibian Rooms at the Henry Doorly Zoo; model for Honduran facility.

