

Conservation of the Critically Endangered Green-eyed Frog *Lithobates vibicarius*

Project report: by Mark Wainwright and Andrew Gray.



TABLE OF CONTENTS

1. Background
2. Preliminary project aims
3. Timeline of activities, aims completed, site visits and preliminary evaluations.
September 2007 – December 2008.
4. Preliminary Chytrid results and evaluations
5. Overview and future of the project

Background

The breeding site of the Critically Endangered frog *Lithobates vibicaria* at Chutas, Monteverde



Lithobates vibicarius is a critically endangered frog species that was once abundant at several high altitude sites in lower Central America, including the area of Monteverde, Costa Rica. Here, the population crashed in the 1980's and, until recently, the species was thought to have disappeared completely. Mark Wainwright, a highly respected local Costa Rican naturalist and conservationist, rediscovered the species at Monteverde in 2005. What is thought to be the last remaining viable population for the species is now known from the one breeding pond, named Chutas, at Monteverde.

In September 2007, Andrew Gray from Manchester University accompanied Mark Wainwright in the field to jointly assess the breeding site. Following the heaviest downpour of rain all year, a landslide, and a very difficult trekking environment, they travelled to El Valle (the midway camp) and then on to the Chutas site. They were well supported in the field by a guard from the Monteverde Conservation League, Geiner Alvarado, who proved particularly interested in the project and who was a very valuable asset. At this time a very large aggregation of breeding *L. vibicarius* was witnessed. The pond contained thousands of eggs and tadpoles at various stages of development. The Directors of The Monteverde Conservation League, The Tropical Science Centre, and also the Costa Rican Ministry proved highly supportive of the fieldwork and also facilitated the collection of a small number of tadpole specimens in the hope that an *ex-situ* captive Ark could be established (Collection and Export Permit Number:DGVS-1148-2007).

Upon his return, Andrew Gray worked on a proposal to establish a preliminary conservation project that included both *in-situ* support and *ex-situ* action. Recognising that successful conservation necessitates collaboration and specialisation, the initial multi-disciplined project would rely on the support of specialists, committed individuals, and institutions. To get the project quickly off the ground, and to help facilitate the promise of a larger ongoing project, a team of key collaborators contributed their time, energy and resources to conducting this valuable preliminary project.

Chester Zoo adopted the conservation project and fully supported fieldwork, monitoring, and sampling throughout 2008 to date. Had it not been for Chester Zoo, this preliminary project would not have been possible. Recognising the importance of the project, Dr Mathew Fisher of the Imperial College kindly offered to process all samples and test for chytridiomycosis. It must be noted that all individuals involved have contributed valuable time and personal resources to this preliminary project.

Preliminary Project

A preliminary conservation project having a holistic approach was proposed in the hope that the following aims might be met and that this would lead to a future collaborative project between the institutions involved.

AIMS of the preliminary project:

- Support local conservationists to work with the species.
- Bring potential collaborators together to share and develop a common conservation goal.
- Initiate regular monitoring of the fragile population at the breeding site.
- Initiate an assessment of the health status of the wild population, particularly regarding the early detection of Chytridiomycosis.
- Collect preliminary data on the specific environment of the locality for the first time.
- Raise local awareness of the project, its importance, and of Costa Rican amphibian conservation in general at Monteverde.
- Raise awareness of the plight of the species, and of Costa Rican amphibians, globally, or to as wide an audience as possible.
- Establish the professional and demographic captive management of the species. (Something now urged for by the IUCN and echoed in the Global Amphibian Conservation Assessment Plan (ACAP).
- Contribute to innovative research on Chytridiomycosis.
- Develop protocols for the collection of related data by local personnel (park rangers), as apart from protecting the area from intruders, the importance of monitoring the health and population status of wild frogs on an ongoing basis at the site at Monteverde will be crucial.

Timeline of activities, aims completed, site visits and preliminary evaluations

February 2008:

Mark Wainwright met with the director of the Children's Eternal Rainforest / Monteverde Conservation League (Carlos Munóz) in order to explain the project and get permission to camp and work in the area. Carlos was presented with a written project outline and an explanatory letter that AG sent from England. MW also met with the director of the Monteverde Cloud Forest Preserve (Carlos Hernández) to explain the project and gain permission to use El Valle cabin on the way to and from the *vibicarius* site. The cabin had been officially closed and off limits for more than a year. Both directors expressed their full support of the project, and neither requested the payment of any of the fees that would normally be required for such work.

All chytrid water sampling equipment was sourced by Stephanie Dawson, purchased in England and sent out to Mark Wainwright in Costa Rica via courier. SD liaised between MW and Mat Fisher to ensure all equipment was correctly sent and samples arrived safe. She also liaised to ensure all procedures to be carried out in the field were clearly understood and recommendations could be adhered to. Data loggers (to measure both temperature and humidity) were organised, programmed, and sent by Douglas Sherriff direct from Chester Zoo to Costa Rica. Unfortunately, Costa Rica's archaic customs offices retained the equipment (without sending any notice to sender or receiver), causing a several week delay - as well as a tax fee of \$50. MW was thus obliged to cancel the planned first trip in March. With the trip delayed, a new pair of data loggers programmed to collect data during the correct time frame had to be sent from England. Like the first pair, these were retained by customs and taxed, despite the accompanying explanatory letter from AG.

May 2008: Fieldtrip 1

With the customs/datalogger problem finally resolved, MW re-scheduled the first site visit under this project for early May. However, conditions were still very dry in early May this year - colleagues of MW who passed by the breeding site at this time reported that the main pond was almost completely dry. Since *Lithobates vibicarius* apparently breeds explosively only after the heaviest rains, MW felt that the trip would be more productive if postponed until after the arrival of consistent rains. The first heavy rains came in the last week of May, and MW led a 5 day trip out to the site from May 30th to June 3rd. He was accompanied by herpetologist Evelyn Cassares, as well as Carlos Suárez and Dennis Corrales. All three are employees of the El Ranario, an exhibition center in Monteverde that teaches tourists and students about local frogs. The team hiked to El Valle cabin on the afternoon of May 30th, then on to the *vibicarius* site on the 31st. The team returned to El Valle on June 2nd and hiked out on June 3rd. MW used grant money to provide all food for the trip, and loaned much of the camping and cooking equipment. MW also gave the team an informal but lengthy lecture on the theme of frog declines and the relevance of this study to understanding declines. He also passed on the sampling protocols that had been sent by Stephanie Dawson, and explained how to distinguish the area's most commonly encountered frogs and their calls.



May, 2008: As hoped, the pond had filled, and *Lithobates vibicarius* was out and breeding. Several hundred frogs were observed in and around the main pond at this time, the majority in amplexus (Photo taken at the most active pool on the morning of June 1st)

The densest aggregations of frogs were in a handful of small pools behind the main pond, as they had been when AG and MW witnessed another breeding bout in August 07. The team camped close to the site on the nights of May 31st and June 1st. Breeding (and calling) activity appeared constant, day and night, throughout the period the team was in the area, from the afternoon of May 31st to the morning of June 2nd.

During this period, the team installed two data-loggers adjacent to two of the satellite pools where *Lithobates vibicarius* was active. The team took water samples from both the main pond and three of the satellite pools. And the team swabbed a sample of *Lithobates vibicarius*. During their work, the team encountered about 10 dead adult *Lithobates vibicarius*, or pieces thereof, and some of these were also swabbed. No other species were encountered or heard at the main pond, but the team swabbed a few other species encountered along the trail. Many of these other species were encountered during a night census conducted along the main trail on the night of May 31st. A prolonged and heavy electrical storm on the afternoon and night of June 1st interrupted field work (as well as flooding the tents and campsite).

September 2008: Fieldtrip 2



Several members of the team at Monteverde

Several UK members of the project team travelled to Costa Rica for meetings concerning the development of the project and to support fieldwork and data collection. On the 5th of September, AG, RG, DS, SD, and MW met with the new director of the Monteverde Conservation League, Yúber Rodríguez, and with the potential project coordinator for the MCL, Mia Roberts. On the 6th of September, the same group met with the director of the Monteverde Cloud Forest Preserve, Carlos Hernández, the Reserve's director of investigation Alan Pounds, and investigation coordinator Yorineth Méndez. Discussions were led by Richard Gibson regarding the development of a collaborative, longer-term project. The meetings showed great promise for future collaboration in amphibian conservation, and for working together to develop a larger project in the future.

MW coordinated permits, food, and other logistical preparations for the field trip, and the whole group hiked to El Valle cabin on the afternoon of Sept. 7th. The MCL and the TSC again waived all fees, and on this occasion provided park guards to help with and learn from the expedition. On September 8th, Mark Wainwright, Douglas Sherriff, Stephanie Dawson, and park guards Geiner Alvarado and Luis Solano of the MCL and Luis Obando of the TSC continued on to the *vibicarius* site, arriving at about 3 pm. Although there was no breeding activity at the pond, the team did encounter dozens of juvenile *L. vibicarius* as well as a few adults. The team swabbed a sample of these, as well as other species (mostly *Craugastor podiciferus*) encountered along the trail. Geiner Alvarado was able to provide GPS coordinates for all the sample locations on this trip. The team also replaced the pair of data loggers that had been installed in June and took more water samples in and around the pond. DS also took PH readings in and around the pond. All samples were taken back to England by DS.

MW provided the guards information on frog declines, this project, and frog identification techniques. SD, DS, and MW all participated in showing the guards protocols for sampling, a task they took to with impressive enthusiasm and ability. The guards became so eager to find frogs (even after the gruelling hike and a rainy late afternoon and evening spent setting up tents and a cooking area, digging drainage ditches to prevent flooding, and clearing a new tent site with machetes) that they went on an independent several-hour long excursion to look for frogs late at night. All expressed great interest in participating in such work in the future.

October 2008: Amphibian workshop for guards

In response to the enthusiasm of the guards who participated in the September field trip, as well as to the clear aim of Richard Gibson to involve the MCL and the TSC more directly in the project for at least the following year, MW set up a two-day amphibian workshop for employees of the two organisations. The workshop took place on the 20th and 21st October at the San Gerardo Field Station, which is owned by the MCL. Each organisation selected 5 employees, who were asked to make a small contribution to cover food costs. The MCL did not charge anything else for use of the station, and MW did not charge anything for leading the workshop. The group did two extensive night hikes that covered from 1200 m to 1700 m in elevation on the Caribbean slope of the Children's Eternal Rainforest. MW provided identification techniques and natural history information for all amphibian species encountered and heard during the hikes. During the daytime, MW gave a series of powerpoint lectures on amphibian taxonomy and natural history, Monteverde amphibian species and their calls, the natural history of frog calls, and on frog declines. The latter concluded with discussion of *Lithobates vibicarius* and the role of this study.

The entire workshop was conducted in Spanish. For most participants, this workshop provided their first opportunity for serious education about amphibians: few had received formal education beyond the secondary school level, and literature on Costa Rican amphibians published in Spanish is scarce. Yúber Rodríguez, the new interim director of the MCL, reported recently that he was thrilled to see that, since the workshop, his guards have not missed an opportunity to go "frogging". MW notes that the guards roam these protected areas more than anyone and can thus contribute valuable observations on Monteverde's ever-changing frog fauna.

December, 2008: Fieldtrip 3

For this expedition, MW was joined by MCL park guards Luis Solano and Brayan Artavia. Both had attended the amphibian workshop. Luis had also participated in the September field trip. The team hiked to El Valle cabin on December 12th, out to the *vibicarius* site and back to El Valle on Dec. 13th, and out from El Valle on Dec. 14th. This period in the year (mostly December to January) is Monteverde's "windy-misty season", when northeasterly trade winds drive fine mist and rain over the cloud forest. This weather pattern held prior to and throughout the trip, creating cold, wet, and muddy conditions that were quite challenging. Treefalls are common at this time of year, and the team had to machete through or around several of them on their way to the site. The *vibicarius* pond was full but the satellite pools had little or no water in them and there was no evidence of breeding activity. The team encountered only one *L. vibicarius*, an adult in the vicinity of the breeding pools that had two leeches attached to its body. This individual along with other species encountered along the trail were swabbed, the data-loggers were replaced, and water samples were taken in and around the pond.

Preliminary Chytrid (Bd) results.

The first samples (28) were collected in the field and sent to Matt Fisher on June 11th, 2008. The preliminary results (see appendix) indicated that chytrid was indeed present at very low level at the Chutas lake site. From the samples it was found that three animals were positive for Bd. All three were dead when the samples were collected from them, and all were infected (presumably these females may have died from being stressed/drowned due to amplexant males during breeding). The infections on these were pretty low (0.001, 1.29 and 0.2 zoospore-equivalents). All the other animals were negative. These results suggest that although chytrid is present at the site, under natural, unstressed, conditions these frogs may remain healthy and unaffected by the fungal pathogen. However, at the moment we can only conclude that: Bd is present in the region; Bd is associated with mortality (but this is not proof that Bd killed these animals; Live animals, if infected, are infected at levels that are undetectable by PCR.

Overview and future for the project.

This preliminary project has proved extremely valuable in initiating *in-situ* conservation efforts towards supporting *L. vibicaria* and in developing close collaborative links to further a larger project. It has facilitated regular monitoring of the wild population and the collection of new and important data relating to health assessment of the population and the specific environment at the site, the collection of which has fully involved local people and staff at Monteverde. It has also helped develop protocols for the collection of future data by local personnel. The preliminary health assessment of the wild population indicates that Bd is present, but also that the species seems capable of surviving this at its present level. The ongoing collection of new environmental data from the site has also been facilitated as part of this project but this aspect is too early to be evaluated and put into context. Through high profile media coverage and local personnel training exercises, this preliminary project has raised much local and global awareness concerning amphibian conservation at Monteverde. Associated media coverage has also highlighted Chester Zoo's involvement in supporting *in-situ* amphibian conservation in Costa Rica. As well as providing necessary *in-situ* support, a viable *ex-situ* population of the species also now exists at Chester.

Building on the success of the preliminary project, the importance of monitoring the health and population of wild *Lithobates vibicarius* on Monteverde Conservation League land, on an ongoing basis, will be crucial. Several aspects require further work, including modification to monitoring, further disease investigation, further training of field staff, and the collection of further complementary environmental data. A study of the population dynamics of this species would be also very useful in developing our understanding, so this can be used help in conserving it. From maintaining the species in captivity, we now know that the males are highly territorial. However, we currently know nothing of their wild ranges or indeed dispersal patterns from the site. We also know nothing of the genetic diversity of the population at the site. One major problem for small populations is that they have lower genetic diversity. This will make them prone to suffer from inbreeding depression and, by chance, they can also lose gene variants that they need to adapt to environmental change. It is therefore important to determine how much genetic diversity a population has to enable the necessary conservation measures to be put into place to conserve this diversity for the long term survival of the species. DNA collection would be through non-invasive means (mouth swabbing).

Lastly, we would hope that future work would involve supporting fieldwork to investigate other locations where this and other critically endangered Costa Rican species may also naturally occur in an effort to confirm their conservation status. We hope to the future of the project will be supported and propose to continue working together in partnership to help support this and other associated Costa Rican amphibians at Monteverde.

Acknowledgements:

We would like to thank all the people that have made this preliminary project possible, especially all the contributors and collaborators listed in this report. We would also like to thank Matt Fisher and Chester Zoo for their involvement and support of this project.