TERRA INCOGNITA

TOAD CONSERVATION PROJECT OVERVIEW: PUBLIC NOTES

Notes from the Arizona Field Study, **August 2017** http://www.terra-incognita-project.org/toad-conservation-project/

PROJECT OBJECTIVES & OUTCOMES

We acknowledge that any long-term conservation program for *Incilius alvarius* must address the multiple factors contributing to global amphibian decline as well as local issues affecting the toads across the entirety of their range including:

- Habitat loss
- Environmental contamination/pesticides
- Pathogens (specifically amphibian chytrid fungus, Bd)
- Climate change
- Overharvesting/poaching and other human impacts

This pilot study addressed these issues by meeting the following objectives:

- Gathering baseline health, distribution and breeding data from Arizona study populations
- Developing proper handling techniques that minimize risks to toads
- Strengthening stakeholder partnerships to support conservation efforts
- Reviewing existing research, analyzing data and reporting findings
- Identifying future priorities and informing long-term research design

Because the resilience of toads populations is dependent on healthy, high quality breeding sites, we identified sample areas in Arizona and mapped potential breeding sites (including seasonal and permanent pools, irrigation ditches and stock tanks) during the August of the 2017 monsoon-breeding season. These may serve as sampling areas for future research. In addition, we analyzed breeding sites for presence of chytrid fungus (Bd) via non-invasive skin swabs (Boyle et al, 2004) and water samples. This will help to establish baseline levels of Bd infection in our sample sites and to cross-reference with other mapped sites by other bodies.

During our pilot study we developed (in concert with herpetologists) and practiced proper handling protocols that minimized disease and stress risks to protect toads from Bd infection via human handling (see Toad Best Handling Practices document). These protocol “best practices” recommendations are available for groups or individuals who seek to sustainably gather toad secretions, where it is legal to do so. We understand the political and social implications of this document and we do not in any way condone or encourage more milking of the Bufo Alvarius toad in the Sonoran region. But people are engaging with the toad in both Mexico and Arizona, and if so, we hope these protocols can encourage ethical handling and sustainability of toad populations.

These protocols may also be adapted for use in any future captive breeding efforts or establishment of sanctuary sites that receive toads that were previously held in captivity and may be infected with Bd. We met with stakeholder groups including private landowners, public agencies, academic institutions and tribes to further the partnerships and relationships necessary for long-term, landscape-level conservation of the toad and its habitat, and hope to continue this relationship building in the future.

REPORT

Members representing Terra Incognita traveled to Arizona in **August, 2017** to initiate a conservation study of Bufo Alvarius toads in the area in concert with local community members.

Location of Bufo Alvarius toad populations was essential to our data collection overview and to developing relationships with existing members of the local toad community was our first objective. Initially, our only on the ground contact was a local woman from Phoenix – KA, who was our liaison to more populous sites of Bufo activity within the Tuscon area and more south towards the Mexican border. There were cultural and personal sensitivities to be navigated with her shepherding members of our team and being concerned about giving out information on toad sites and how to conduct travel and monitoring.

To help assuage sensitivities we agreed that toad sites are sacred and privileged information and we, the members of the **Terra Incognita** *Toad Conservation Project,* promised to keep confidential all information around toad sites that are shared with us and have all interested parties sign binding non-disclosure agreements. Our non-disclosure agreement can be found here: TCP-non-disclosure-agreement.doc

Note that:

- Affiliates are undertaking their own missions to go out and find samples of the Bufo Alvarius/Bufo Incilius toads in Arizona. They take full responsibility for their actions and have secured all licenses for such activities (AZ fishing/game licenses etc.).

- Terra Incognita and the Toad Conservation Project is working with existing affiliates to gather relevant information on Bufo toads locations, habitats, health and other data. Terra Incognita is not engaging in any milking of toad venom or any illegal activities.

- A TCP affiliate was in charge of swabbing and taking a DNA sample from toads to check for chytrid virus and collecting that sample in a sterile vial for later testing

- GPS coordinates were logged where able, but in the field practice showed that cell phone/data range sometimes lagged and a centralized and agreed form of logging coordinates is needed. Some affiliates used Herpmapper (https://www.herpmapper.org) which is a whole established system for logging amphibian locations, but is owned by a third party whom we may not want to disclose our data to; or www.maps.me which is an app you can download to your phone and record GPS data and other info, which also works offline without data access.

- Most sites studied for toad populations and chytrid fungus were simply screenshoted with an iPhone MAPS program to be cross-referenced with data collected from these sites. Note: temperatures on the screenshots are in Celcius C. An internal data map should be created and cross-referenced with the historical Bufo toad sightings map gifted to us by our consulting herpetologist: alvarius-data-iDigBio.xls (not online).

Data collection between 7/30 and 8/6 was initially sporadic, examining first urban locations around Tuscon itself where first hand reports of Bufo toads were reliable. The monsoon rains had fallen briefly approximately two weeks before and for the full two weeks from 7/30 through 8/14 while some rains occurred, and there was flash flooding alerts around Tuscon in general, there were no big downpours to bring the toads out. This meant that in the urban and isolated environments our contacts knew of, few or no toads were found, even in locations that had previously been plentiful.

A word here about locations: An acquaintance into toad medicine had been in Mexico in the Sonoran desert around Hermosillo and the pueblos exactly a week before our Tuscon trip, and seen hundreds of toads at multiple locations out and breeding. The rainfall situation was the same, the location is only a hundred miles away, although it also must be pointed out that rain and weather conditions vary a lot from one side of Tuscon to the other and in parts of Arizona. There may be storms and flash flooding in the east and nothing in the West of the same city.

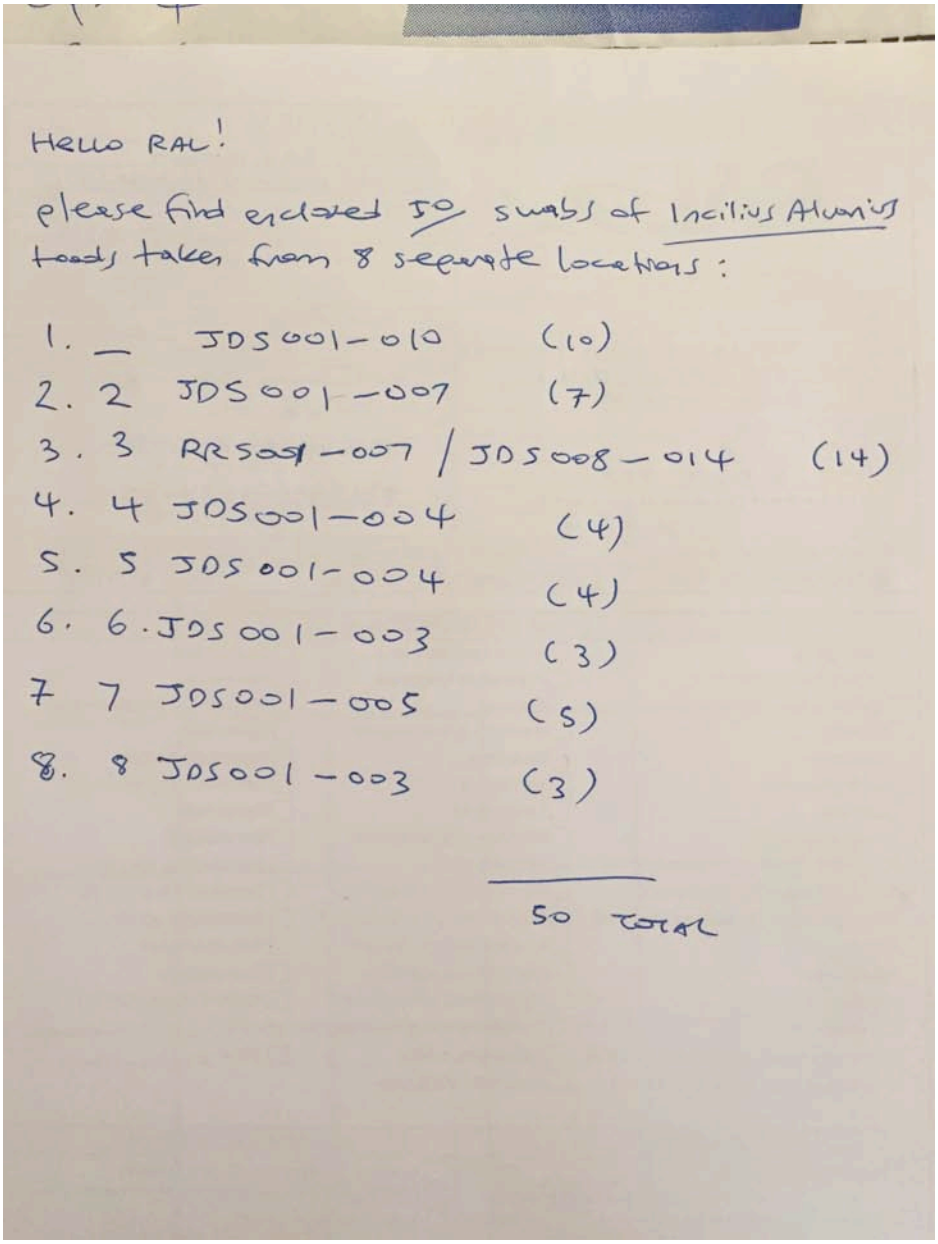
But in general and this is from first hand experience, on the Mexican side of the border of the Sonoran desert in the pueblos and towns, the Bufo Alvarius toad was present after the monsoon rains in late July in great numbers. Whereas in general around Tuscon at the most we found 20 toads at rare live sites, just that week before in the Mexican Sonoran desert, literally hundreds of Bufo toads were found. I would say that there was at least a 10 to 1, probably more like 50 to 1 ratio of Bufo toads in the Sonoran desert versus in random rural areas around Tuscon.

Whether this is due to population development, pesticides, climate, etc. is unknown. At almost all the Tuscon sites we visited there were little to no insects to provide food coverage for the toads, nor were there lights to attract the insects except the urban areas. The edges of the shanty town pueblos around the Sonora may just have the right combination of desert, town lights and insects to attract the population up from below ground. Further thoughts on using light to attract the Bufo toads can be found in our prototype Toad Sanctuary document. (forthcoming).

During our second week of investigations we partnered with more experienced local toadsters from the community who agreed to take us out to established sites where Bufo toads were known to inhabit each year. This greatly established trust and connection with these affiliates, all of whom had relevant fisheries and wildlife toad licenses to handle toads. We were able to explain our best practices document and to make sure all toads handled were done so with latex gloves when handled at each site to stop any potential transmission of the fungus. Swabbing was done in accordance with best practice protocols to ensure toads were not harmed. Said affiliates agreed to work as ‘citizen science’ representatives of the Toad Conservation Project in future seasons and to collect metric data on populations, climate etc.

This second week was much more fruitful and resulted in a collection of eight sites where Bufo toads were sighted and approximately 50 chytrid fungus swabs being tested on Bufo toads at these sites by the local affiliate partners. A list of sites and toad populations found and results of the chytrid fungus testing are held confidentially by TI.

CHYTRID FUNGUS RESULTS



Hello RAL!

please find enclosed 50 swabs of Incilius Alvarius toads taken from 8 seperate locations:

1. _ JDS001-010 (10)
2. 2 JDS001-007 (7)
3. 3 RRS001-007 / JDS008-014 (14)
4. 4 JDS001-004 (4)
5. 5 JDS001-004 (4)
6. 6. JDS001-003 (3)
7. 7 JDS001-005 (5)
8. 8 JDS001-003 (3)

50 total

Batrachochytrium dendrobatidis
COMMON NAMES: CHYTRID FUNGUS, CHYTRIDIOMYCOSIS

ORGANISM: Chytrid is an infectious fungal disease of amphibians. This test detects the original forms found in all amphibians, but does not detect the newly discovered chytrid now being found in some salamanders. For the new variant see: **Batrachochytrium salamandrivorans.**

SAMPLE REQUIREMENT FOR TESTING:

LIVE ANIMAL TESTING: Dermal swab or tissue.

POSTMORTEM TESTING: Dermal swab or tissue

ENVIRONMENTAL TESTING: Sterile swabs from immediate housing area, cages and enclosures can be diagnostic. Additionally, swabs collected during or after clean-up efforts can be useful to determine proper disinfection of contaminated areas.

SPECIES INVOLVED: Amphibians.

ZOONOTIC POTENTIAL: ***NO***

ADDITIONAL INFORMATION: Testing performed daily. qPCR allows monitoring of disease status, efficacy of treatment or therapies and clean-up efforts.

EXPLANATION OF RESULTS:
Positive results confirm target organism contained in sample(s) submitted. Negative results confirm absence of target organism in sample(s) Occasionally we will report a sample as "HOT" to reflect the extremely high level of target organism present in sample. In cases of zoonotic potential, extreme care should be taken when housing, handling and disposal.

TESTING METHOD: Quantitative Real Time PCR (qPCR). Test is performed daily with results emailed same day (if received in Lab before 12:00 noon) Monday through Saturday.

Real Time PCR Testing vs. PCR Testing

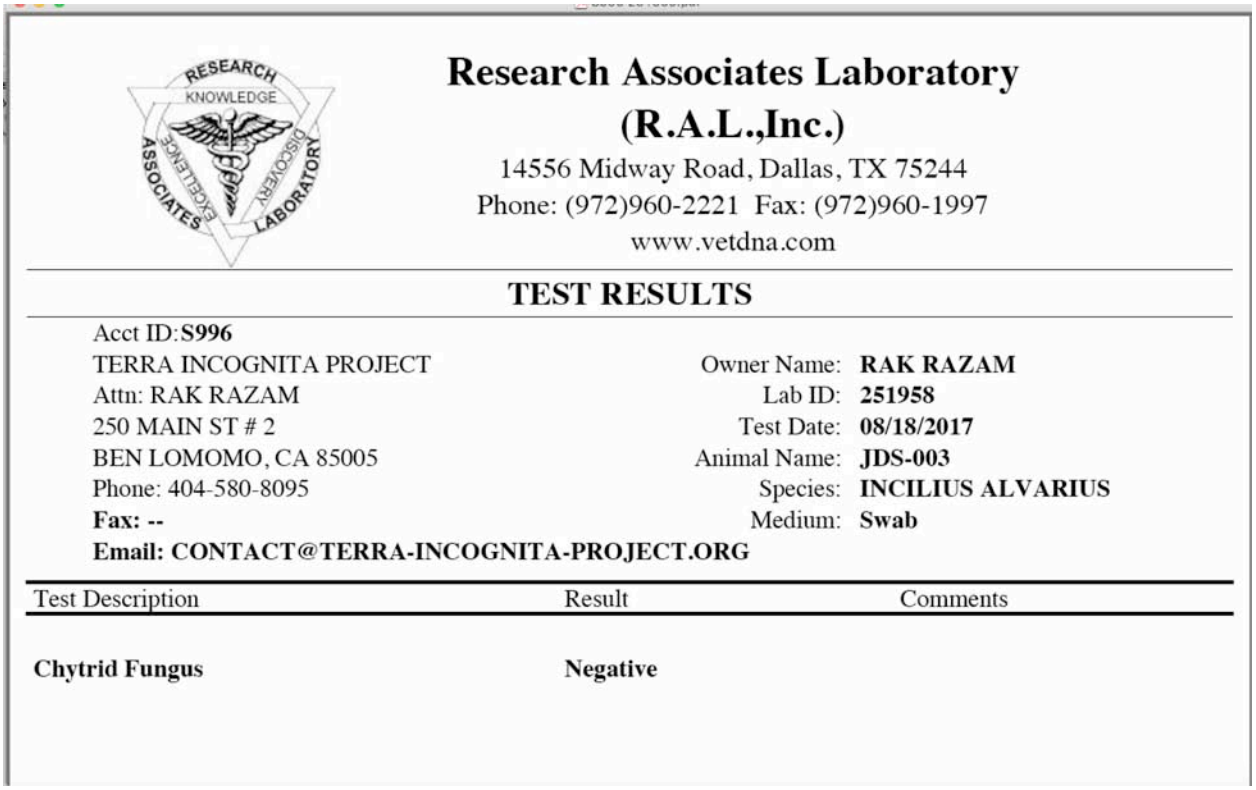
Most people are familiar with PCR technology and its advantages. Since 1992, R.A.L., Inc., has been performing avian diagnostics utilizing standard PCR methods. With the relocation of our laboratory to Dallas, Texas, in 2002, we took the opportunity to upgrade our equipment and services. These changes brought many improvements in quality, services and accuracy. Perhaps the single most important change has been new state-of-the-art equipment and associated technology called ***Real-Time PCR***. ***Real-Time PCR*** is the most recent advancement to PCR technology and has many advantages.

For the first time, we have the ability to actually quantify the amount of pathogen present. This ability has been both long overdue and sorely needed. Samples are compared to known positive controls on each and every cycle. With standard PCR, your results are only positive or negative. A low level measured by standard PCR may or may not be detected, depending on the sensitivity of equipment and technology, as well as technician skills.

If detected and reported as positive, veterinarians and clients have no starting point and can only envision the worst-case scenario. If the same sample is reported as negative, there is a false sense of security, as this animal may still be an intermittent shedder.

Real-Time PCR allows us to compare the variable states of infection. By knowing where within the disease process these animals are can help determine a plan of intelligent action. Also, in the event of an outbreak, ***Real-Time PCR*** can effectively monitor the success of clean-up efforts.

Real-Time PCR monitoring reduces possible contamination, provides results more quickly and saves technician time. All this adds up to greater sensitivity, faster results and cost savings that are passed on directly to you! Thanks to Real-Time PCR and volume, R.A.L. now performs testing six days per week with up to three runs daily. Samples sent overnight can be tested and reported back to you the same day they are received in the lab. All results are faxed back within 24 hours, and hard copies are also mailed, all at no additional cost! New technology and great clients make for a successful and growing industry. We thank you for your ongoing support and look forward to many more successful years in the future.



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TEST RESULTS

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Owner Name: **RAK RAZAM**
Lab ID: **251958**
Test Date: **08/18/2017**
Animal Name: **JDS-003**
Species: **INCILIUS ALVARIUS**
Medium: **Swab**

Test Description	Result	Comments
Chytrid Fungus	**Negative**	

OVERVIEW:

A total of 50 swabs were conducted on Bufo toads across eight sample sites around Tucson, AZ, with no chytrid fungus found overall. As the information below outlines, while our grassroots study of eight sample sites was limited it showed geographical diversity and negative results for the chytrid fungus in the small populations of Bufo we sampled. Still, most of the historical chytrid samples found in Bufos have occurred in West AZ in areas we did not cover due to logistical issues. Our pilot study addressed the following objectives:

- Gathering baseline health, distribution and breeding data from Arizona study populations
- Developing proper handling techniques that minimize risks to toads
- Strengthening stakeholder partnerships to support conservation efforts
- Reviewing existing research, analyze data and report findings
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And in this regard all our objectives were targeted and successfully implemented. From a cold start we have developed firm relationships with local toad community members, enlisting them into our Citizen Science program to gather toad population data in future years with proper AZ fisheries and wildlife scientific licenses. We have gathered health and distribution of population of Bufo data, and assessed the difficulties and variables on the ground with rainfall, seasonal changes, and climate.

We have taught and disseminated best practices techniques and handling practices to stop the spread of the chytrid fungus, as well as milking to ensure the immune system of the toad is given time to regenerate its venom and stay protected and viable and to ensure the toad experiences the minimum stress in handling. Complex legal and ethical issues have been successfully navigated and now we envisage these protocols may also be adapted for use in any future conservation work with the bufo toad.

We have also established relationships with professional herpetologists working with the Arizona Fisheries and Wildlife department who can advise us on moving forward and overviews for integration of these learnings. We have also been gifted with a XLS file of over 100 historical sightings of the bufo toad in the AZ region that need to be revisited and logged for current populations, and we have engaged our citizen science conservation members on the ground to be part of this as we extend and continue our conservation efforts in the 2018-19 season.

For more information see: http://www.terra-incognita-project.org/toad-conservation-project/ And to support ongoing Bufo Alvarius Conservation:

TERRA INCOGNITA PROJECT is a 501c3 Not-for-profit corporation founded in California, Sept 9, 2015. EIN number: **47-5001833. Your gift is tax-deductible.**
TO DONATE: contact@terra-incognita-project.org for information on how to make a direct bank deposit. Make checks payable to 'Terra Incognita Project' and mail them to:
Terra Incognita Project/250 Main Street, Unit 42, Ben Lomond, California, 95005