

INFORME FINAL

FINAL REPORT



Análisis de la Viabilidad de la Población y del Hábitat Del Sapo Concho Puertorriqueño

Population and Habitat Viability Assessment
for the Puerto Rican Crested Toad

(Peltophryne lemur)

27 - 29 October 2003
Boquerón, Puerto Rico





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Del Sapo Concho Puertorriqueño**

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A contribution of the IUCN/SSC Conservation Breeding Specialist Group.

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Population and Habitat Viability Analysis for the Puerto Rican Crested Toad

27-29 October 2003

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**Population and Habitat
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**SECTION 1
Letter of Invitation**

Letter of Invitation



AMERICAN ZOO AND AQUARIUM ASSOCIATION

DATE: August 27, 2003

RE: Puerto Rican Crested Toad PHVA, October 27-29, 2003

Dear Participant:

On behalf of the Fish and Wildlife Service (FWS) and the American Zoo and Aquarium Association's (AZA) Puerto Rican Crested Toad Species Survival Plan (SSP) program, we invite you and/or members of your staff to participate in an IUCN Conservation Breeding Specialist Group (CBSG) **Puerto Rican Crested Toad Population and Habitat Viability Assessment (PHVA)** meeting.

This CBSG meeting provides an opportunity for Fish and Wildlife Service (FWS) and the Species Survival Plan (SSP) program to assess population viability and identify priority objectives for the recovery of this threatened species. The priority actions agreed to at the meeting will provide a framework for new in situ conservation, education and recovery projects. Your participation will ensure that we have data from those who have field or research experience with the crested toad, *Peltophryne lemur*. Data from this meeting may also be used to review Recovery Plan objectives and status designation. After the meeting, the SSP will be participating in projects identified as priorities at previous meetings. These include surveys for additional sites for re-introduction of tadpoles; a working team assisting the Juan Rivero Zoo in developing displays and outreach resources for species at risk; and assisting Guanica Forest Manager, Miguel Canals, in breeding beach projects. We have coordinated the meeting dates to coincide with the release of zoo bred tadpoles this year and some zoo staff will be monitoring toadlets as they emerge from the breeding ponds.

Please join us at the FWS Caribbean Field Office, Boquerón, Puerto Rico for the PHVA meetings October 27- 29, 2003. We are planning a site visit/field trip for Sunday, 26 October, so please plan to arrive in San Juan early on Saturday, 25 October. Some participants will be arriving earlier or staying later as part of working teams. Confirm your attendance by contacting bjohnson@torontozoo.ca, by FAX: (416) 392-4979 or by mail: Bob Johnson, Toronto Zoo, 361A Old Finch Ave. Scarborough ON Canada M1B 5K7

Whether or not you will be able to join us at Boqueron, we value your expertise and knowledge. We will be compiling a "briefing book" of background materials which might be useful at the workshop, and we would appreciate any suggestions or contributions you can make to the "briefing book". This would include: reprints and manuscripts of scientific papers, reports from field studies or reports about the captive breeding programs, and any other documents that will help to provide context, background, or data that will help us to make more complete analyses and more informed decisions. Please send anything you have to the CBSG office (12101 Johnny Cake Ridge Road, Apple Valley, MN, 55124, U.S.A.; e-mail: office@cbsg.org) before 1 October.

We look forward to your contribution to the recovery of this threatened species.

Sincerely,

Bob Johnson,
AZA Puerto Rican crested toad SSP Co-ordinator.

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**SECTION 2
Agenda**

PHVA Agenda

Sunday, October 26th Site visit: Guanica Forest
 Juan Rivero Zoo, Mayaguez, Exhibit Design

Monday, October 27th

09:30	COFFEE	
10:00	Introductions and PHVA workshop goals: Why are you here?	CBSG, B. Lacy USFWS, C. Diaz SSP, B. Johnson
10:20	USFWS recovery goals	Silmarie Padron
10:40	Breeding history of Sapó Concho, Guanica Forest	Miguel Canals
11:00	BREAK – questionnaires	
11:15	Field Research, Sapó Concho, Guanica Forest habitat assessment	Jaime Matos
11:35	Video	Miguel Canals
12:15	LUNCH	
13:15	Breeding history of Sapó Concho, Quebradillas	Ernesto Estremera
13:35	Status of captive population and SSP goals	Bob Johnson Andrew Lentini
13:55	Discussion: key issues for Sapó Concho conservation: Individually, then group, then prioritize	Bob Lacy Bob Johnson
14:30	BREAK	
14:50	Introduction to population viability analyses: Vortex models	Bob Lacy
15:15	Factors affecting the toads and their impacts on populations projections: building a model	Todos
16:00	Working Groups I: Data available/assumptions/data needed related to key issue	Grupos
16:30	End of day 1	

Tuesday, October 28th

09:30	COFFEE	
10:00	Conservation options for Sapó Concho	Todos
10:30	BREAK	
10:50 – 12:00	Working Groups II: Elaboration of options	Grupos
12:00	LUNCH	
13:00	Group reports and discussion: building scenarios	Todos
14:30	BREAK	
14:45-16:30	Working groups: developing solutions	Grupos
19:30	DINNER & DISCUSSION: La Paguera	

Wednesday, October 29th

09:30	COFFEE	
10:00	Group reports and recommendations	Todos
11:00	BREAK	
11:20	Actions and resources required for Sapó Concho conservation	Grupos
12:30	LUNCH	
13:30	Responsibilities required for Sapó Concho conservation	Todos
14:30	BREAK	
14:50	Status designation, Recovery Plan and Action Plan	USFWS – Todos

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**Section 3
News Releases**

News Release

For immediate release

Contact: Tom MacKenzie, 404/679-7291
Silmarie Padron, 787/851-7297

Exotic Sapo Steals Name!

Puerto Rico's only native toad is called "sapo concho." The sapo concho puertorriqueño (Puerto Rican crested toad, *Peltophryne lemur*) was once found in areas of karst from Alta Baja to Aguadilla in the north, and from Coamo to Guánica in the south. After the introduction of another larger toad, the marine toad (*Bufo marinus*), to control the sugar cane beetle in the 1920's, both toads were called sapo concho. Because the native species was rarely seen the name sapo concho was used to describe the more abundant marine toad. Two toads with the same name makes conservation of the rare Puerto Rican toad difficult.

To further increase awareness of the island's unique sapo, scientists will now call the native toad "sapo concho puertorriqueño" and the marine toad will be called "sapo común," or common toad. A media campaign is being developed to help the public understand the importance of the unique crested toad and to distinguish it from the marine toad. Conservationists are asking Puerto Ricans to identify areas where the toad may still be found and report sightings to Silmarie Padron, Fish and Wildlife Biologist at 787-851-7297 ext. 41 or silmarie_padron@fws.gov.

At an international meeting held in Boquerón from October 27 through 29, scientists, managers, educators, and other stakeholders tackled many issues dealing with the conservation of the sapo concho puertorriqueño. The group participated in a workshop to assess population viability and identify priority objectives for the recovery of this threatened species. The priority actions agreed to at the workshop will provide a framework for new management, research, and education projects.

This meeting coincided with the announcement by Guánica Forest Manager, Miguel Canals, that the range of the toad has been expanded. Toads recently reproduced at a protected area that has had captive bred tadpoles reintroduced to Puerto Rico from 21 American and Canadian zoos and aquariums over the past 10 years.

The workshop was facilitated by the IUCN Conservation Breeding Specialist Group, and included representation from the U.S. Fish and Wildlife Service, Puerto Rico Department of Natural and Environmental Resources, American Zoo and Aquarium Association, Compañía de Parques Nacionales (Zoológico), University of Puerto Rico, and educator Mr. Ernesto Estremera.

"This meeting helped the Ecological Service Office in Boqueron PR identify priorities and actions which must be implemented to conserve this species, and most importantly, reinforced communication and collaboration between state agencies, biologists, universities and the

community necessary for the conservation of the sapo concho puertorriqueño” said Silmarie Padron, Fish and Wildlife Biologist of the Boqueron Field Office.

The workshop’s management group emphasized the need to work together with the scientists, the community, and the non-governmental organizations to achieve the goal of protection and conservation of the Puerto Rican crested toad for future generations.

“We need the involvement of all sectors of our society to conserve the sapo concho puertorriqueño. We cannot do it by ourselves,” said Miguel Canals, manager of the Guánica Commonwealth Forest, Department of Natural and Environmental Resources.

“Research concerns include finding and monitoring existing populations, identifying genetic differences between the northern and southern toads and documenting all aspects of the life history that may be important to ensure the survival of this species,” said Dr. Enrique Hernández-Prieto, of the University of Puerto Rico, Humacao Campus.

“The conservation of this unique species is in the hands of Puerto Ricans. Education plays a key role in transmitting information about the sapo concho puertorriqueño,” said Norma Villarrubia, of the Juan Rivero Zoo.

Exciting activities are planned by the workshop’s education group. The Juan Rivero Zoo’s serpentarium is being renovated to celebrate the sapo concho puertorriqueño and other species that are native to Puerto Rico. A new sapo concho puertorriqueño costume character will be unveiled soon and people should watch for posters and educational materials. In addition, opportunities to help conserve the toad will be available.

It is hoped that Puerto Ricans will get to know and appreciate the sapo concho puertorriqueño and recognize its rare and unique place on the island. For more information on this article please contact Tom MacKenzie at 404-679-7291.

Regarding Conservation Status Listing of the Puerto Rican Crested Toad

The current listing is Threatened at all levels. From the discussions and information acquired during the PHVA meeting, the species meets the requirements for Endangered status. Further discussion acknowledged that the level of protection and funding is the same at the federal level whether the animal is listed as Threatened or Endangered. However, the permitting process is different and may hinder zoos' abilities to participate in the captive propagation. DNER does have more power if they list the toad as Endangered. Surveys of other areas to find the PRCTs have not been completed. Given all this and the backlog of animals in need of listing, changing the status of the PRCT is not a USFWS priority at this time.

We are concerned about the precarious state of the animals in the wild. We strongly encourage reassessment of status as soon as (1) resources permit (2) and /or the completion of surveys (within two years), or (3) a dramatic change in the stability or status of the known populations.

Ongoing Communications

Sapo Concho E-Mail Discussion Group

Although much good discussion occurred at this PHVA meeting, and many important recommendations have been made, it is important to maintain the inertia on behalf of el sapo concho puertorriqueño, and to ensure that conservation recommendations are acted upon. An important requirement for continuing our work on behalf of the Puerto Rican crested toad is to maintain a good network of communication among those involved in research, management, and conservation on the species.

One valuable mechanism for maintaining communication is a web-based (e-mail) discussion group. Subsequent to the PHVA meeting, such a Sapo Concho Discussion Group was established.

To subscribe from this group, send an email to sapoconcho-subscribe@yahoogroups.com. Subscribed members can then send messages to the group at sapoconcho@yahoogroups.com. To visit the group on the web (including access to archives of prior messages), go to <http://groups.yahoo.com/group/sapoconcho/>.

Information Point Person -- Punto Focal de Información

At the PHVA meeting, Norma Villarrubia, Biologist at the Juan Rivero Zoo in Mayagüez, offered to serve as the "Information Point Person" to help monitor accomplishment of workshop recommendations and to facilitate communications among those interested in the conservation of the Puerto Rican crested toad. Among the tasks that an Information Point Person should undertake are:

- Keep track of progress on workshop recommendations

Communicate progress, or lack of progress, to USFWS, DNER, CBSG, and other PHVA participants

Help to provide forums for communication, possibly including an email list, a newsletter, and a web site.

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**SECTION 4
Presentations**

Overview of the PHVA Workshop Process

Robert Lacy, CBSG and Chicago Zoological Society

Conservation is difficult

- Species are threatened by many processes
- Threats arise from diverse human activities
- Many threats are highly unpredictable
- Threats are often difficult to stop or reverse
- Threats interact in complex ways
- There are competing needs, interests, backgrounds, kinds of knowledge, and ideas

But we have the resources to succeed

- People with diverse expertise, knowledge, ideas, and access to resources
- Organizations
- Data, knowledge, and experience
- Commitment

IUCN SSC CBSG Conservation Breeding Specialist Group

- Facilitators, not implementers, of conservation
- *We bring to this PHVA meeting*
 - Experience with other species
 - Some tools to assist you with analyses
 - Workshop processes for promoting collaborations by diverse people on complex topics
 - Links to networks of experts
 - Help with compiling and editing documents, formulating plans that lead to action

You bring to this PHVA meeting

- The data
- The ideas
- Critical and open thinking
- Existing partnerships
- Potential for new partnerships
- Responsibility and ability to implement conservation of Sapó Concho

We are diverse (and that is good), but we also agree on some things

- We do not want to lose the crested toad as a part of the natural heritage of Puerto Rico, a part of a healthy natural system, and a unique component of global biodiversity
- Working together, we can secure a safe future for Sapó Concho in Puerto Rico

Human factors influencing our thinking and problem-solving process

- We all have biases and assumptions
- We seek patterns in events

- We choose a pattern or interpretation with limited analysis
- We select data that support our preference and ignore data that disagree with our preference
- We start our analyses with conclusions rather than defining our problems and needs
- It is difficult to evaluate in our heads all interactions in complex problems
- Groups of people are more productive of ideas and more inclusive of options than individuals working alone.

Working Agreement for the PHVA workshop

- All ideas are valid
- Everything is recorded on flip charts
- Everyone participates; no one dominates
- Listen to each other
- Treat each other with respect
- Differences and problems are acknowledged, not "worked"
- Observe time frames

Tasks to get done

- Examine goals
- Review status
- Enumerate issues
- Build model of Sapo Concho dynamics
- List options
- Analyze assumptions, data, scenarios
- Develop solutions
- Define recommendations and plans for action
- Prepare reports and plans for follow-up

Working Group Roles: Each group will need

- **Facilitator** to assure organized discussions and encourage equal participation; to write notes of the discussions on flip chart pages. These flip charts will serve as the "group memory" for the discussions.
- **Computer Recorder** to enter notes of the working group discussions into a computer. (These files will form the basis of the report from the workshop.)
- **Presenter** to present the results of each working group's discussions in plenary sessions.
Assign roles at the start of each working group session.

Role of the Facilitator

- Keep purpose front and center
- Encourage equal participation
- Not a content resource in the group
- Do not evaluate ideas presented
- Keep group memory (flip charts)
- All contributions have equal weight
- Keep deadlines and produce product

*Information Point Person (IPP) – to be appointed at the PHVA
Punto Focal de Información (PFI)*

- Keep track of progress on workshop recommendations
- Communicate progress, or lack of progress, to CBSG and to participants
- Provide a forum for communication (Email list? Newsletter? Web site?)

*Characteristics hended in the Information Point Person (IPP)
Punto Focal de Información (PFI)*

- Habla español
- Está en Puerto Rico
- Tiene e-mail
- Comunica bien
- Conoce todos (o muchos de nosotros, por lo menos)
- Quiere ser el PFI
- Tiene el tiempo hacerlo

PHVA steps to assist in conserving Sapo Concho

- Identifying key issues
- Building understanding: data, models
- Constructing scenarios
- Identifying options
- Assessing data
- Analyzing scenarios and options
- Developing solutions
- Specifying actions

Identifying key issues

- Needs of the toads
- Your needs
- Concerns about these needs
- Concerns about your needs

Dealing with the data around an issue

- What data are available?
- What assumptions are we making?
- What data are still needed?

Elaboration of options

- Describe the conservation option
- How could we make it work?
- What do you want answered about this scenario?

Goals of the 2003 PHVA workshop

Bob Johnson, Toronto Zoo

As SSP Coordinator I see the following as Workshop goals

- Model long term viability of existing wild populations and threats to long term sustainability.
- Determine relationship between captive and wild populations in terms of long term demographic and genetic management.
- Determine what we know about toad populations and information gaps. Develop a long term research programme.
- Locate additional sites for construction of release ponds in the south. Support GIS habitat mapping and ground truthing of potential sites for the re-establishment of toad populations.
- Make best guess estimate of northern toad population status and develop northern monitoring strategy
- Assess status of habitats that historically supported toad populations and determine if they can be secured.
- Develop plan for protecting or purchasing breeding ponds and habitat for re-introduction from captive northern toad populations
- Develop island wide outreach programming and support Juan Rivero Zoo conservation programs (Note: following the PHVA staff from SSP Zoos assisted with the renovation of exhibits and the construction of two new exhibits to house crested toads and other island endemics).
- Ensure ongoing support, and training where required, for in situ conservation programs by a Puerto Rican infrastructure.
- Determine role for a recovery action group for education/outreach, research, and population management with well defined actions and timelines.
- Provide an urgent plan to determine course of action for northern toads – support Ernesto Estremera education opportunities and municipal involvement in protecting flora and fauna in the quebradas of the Quebradillas area; and identify and secure breeding habitat; gather data to conclude on status of northern toad (extirpated?).
- Discuss current status designation and evidence for uplisting. Determine if current recovery plan is still relevant to guide recovery actions given new data and population trends.

Despite the fact that we have had priority projects to guide us for several years perhaps our greatest hurdle has been ensuring accountability for the responsibilities we have all assigned as priority recovery actions. The Puerto Rican crested toad conservation partnership has evolved over many years. During that time while we as a team have developed skills and trusting working relationships, toad populations are still threatened and there many questions remain on the basic biology and population dynamics of this species. Without data on the life history and ecology of this beautiful toad we have struggled to develop management plans that were tested in the field. Adaptive management guided us in our strategy of providing as many tadpoles at the earliest stage of their life history for release to the selective pressures of the wild. We used

models of other anthropogenic habitats in Puerto Rico to provide breeding habitat. Under the guidance of the PHVA process, we have an opportunity now to build on our working relationships to structure recovery action to ensure the sustainability of not only toads but the conservation partnerships that are much needed for threatened populations of Puerto Rican plants and animals and the habitats that sustain them.

Background

The Puerto Rican crested toad (PRCT) was the first amphibian species selected for inclusion in the AZA SSP (1984). The first joint Zoo, USFWS and DNR meeting took place in 1989. This meeting was the first PHVA for an amphibian species for the CBSG PHVA. The two day meeting concluded with the following recommendations:

- Genetic study to characterize northern and southern toads and extirpated Virgin Gorda population
- Maintaining a captive SSP population as a hedge against extinction and as a source of captive bred toads to increase range of wild populations (only one known southern population and four very small ponds with toads in the south).

The SSP funded the second (first was by Lacy in 1988) genetic study of toad populations (Anna Goebel, 1990). As a result, the variation between northern and southern populations was significant enough to require genetic management in captivity of two populations, northern and southern. This has certainly taxed the available resources and space of participating institutions.

Shortly thereafter data from the PHVA was used to develop the USFWS Recovery Strategy for this species (1991). An important step in the development of recovery action partnerships was a joint USFWS and AZA meeting at White Oak, Florida in 1998. The PRCT SSP and USFWS collaboration for recovery was one of several highlighted at the meeting. The meeting itself resulted in a MOU between the USFWS and AZA to further the recovery of threatened species and to share expertise and resources. The SSP focused on education opportunities through posters, toad models, bumper stickers, and a student activity book. This focus was primarily on increasing the profile of the endemic toad which was confused with the more abundant and widespread introduced marine toad (*Bufo marinus*).

PUERTO RICAN CRESTED TOAD CONSERVATION MEETING 1998

In 1998, the first strategy meeting was held and all stakeholders and recovery partners were invited to participate. This meeting provided an opportunity to assemble data on the biology and habitat of the toad from a variety of field locations. Data on population size, the relationship of breeding events to varying rainfall amounts and barometric pressure changes were summarized along with characterization of breeding and non-breeding population ponds in the north. Subsequent to this meeting, a USFWS, DNER, and AZA SSP Conservation partnership (1998) was proposed with an action plan based on Recovery Plan objectives (see Appendix A). The following priorities were established:

- Need for field identification manuals and additional outreach resources
- Reliability of SSP as a source of animals for re-introduction demonstrated

- In addition, Susan Silander (USFWS), Miguel Canals (DNER), and high school teacher Ernesto Estremera were recognized for their contribution to the PRCT.

Renewed focus on breeding northern toads despite the limited founder numbers. Northern population – 6.3 inbreeding = .125. 66% of gene diversity of population in captivity. Why haven't northern bred- Bob Johnson explained that siblings – our decision – may be wrong. Bob proposes “If any northern toads are found, a maximum of 20 be taken into captivity”. Need to put an effort into looking for them where they should be. Discuss pros and cons of above proposal. Need research on survival of releases. Need research on natural history (highest priority).

- Recognition that protection of habitat and field research should be priority for recovery.
- Screening of toads for disease.

This was the first formal training opportunity with the SSP Vet and Husbandry Advisors providing equipment and practical training in husbandry and reproduction of captive toads to be established at the UPR/Mayaguez and Mayaguez (now Juan A. Rivero) Zoo. In addition, a pond was constructed for translocation of toads from Tamarindo (as part of an emergency plan to deal with catastrophic loss of either toads or breeding habitat. Additional sites were surveyed for the construction of breeding ponds.

Priorities:

- Continued monitoring of northern population
- Habitat protection (acquisition?). Working group to determine which is critical northern habitat and what should be done – is acquisition the only option.
- Establish working group (FWS to do by 1999) and they determine timely “acquisitions” of land.
- Develop amphibian monitoring program on “local” level (i.e. when Puerto Rican toads are found (i.e. monitor all species. Possibly determined by budget. Working group to determine. Miguel is in favor of expanding to all amphibians. Ernesto?
- Outreach display – models, graphics – working group
- Manual of identification – working group
- Educators kit – working group
- Signage at field sites
- Working group to determine land ownership and use and best way to protect them
- Study of basic biology and habitat characterization
- Need to determine other lands for release – North and South
- Collection of northern toads – permits. Short term – initiate permit, evaluate tadpoles?, provide costs
- Immediately breed northern toads – only enough for SSP – no release
- Research – disease pathology, genetics, survival of releases
- SSP maximize all breedings of SSP animals

APPENDIX A: USFWS, DNER AND AZA SSP CONSERVATION PARTNERSHIP (1998)

RATIONALE:

Merge SSP Priorities with those outlined for recovery in the PRCT Recovery Plan. In effect AZA Masterplan requirements and education and field conservation goals are merged with those of the Recovery Plan.

Recovery criteria for *Peltophryne lemur* (as listed in recovery plan):

- * establish 6 breeding populations viable for 10 years
- * establish and maintain 5 captive populations as hedge vs extinction

To accomplish these recovery criteria, the following actions are required:

1. Prevent further population decline and habitat loss
2. Propagation for re-establishment in the wild
3. Establishment of 3 northern and 3 southern populations
4. Island-wide education.

1. PREVENT FURTHER POPULATION DECLINE AND HABITAT LOSS

1.2 SEARCH FOR HISTORICAL AND NEW POPULATIONS

ACTION:

Prepare and distribute identification leaflet to schools and social centres in historical range of toad.

ACTION: Use field I.D., manual (photos, drawings), models, posters, and video of toad to assist in identification and to distinguish *P. lemur* from *B. marinus*

ACTION:

Use live *P. lemur* and models of *P. lemur* to distinguish from *B. marinus*

ACTION:

Conduct surveys in both northwest and southwest Puerto Rico (ongoing)

1.31 PROTECT COMMONWEALTH-OWNED HABITAT

ACTION:

Erect toad information graphics and post breeding areas with information on toad and reason for closure of roads/beaches during breeding episodes.

ACTION:

Organize school children to share in projects (to paint posts that protect Guanica beach breeding habitat or plant shrubs) that highlight importance of Guanica forest.

1.32 PROTECT EXISTING HABITAT ON PRIVATE LAND

ACTION:

Encourage landowners to voluntarily protect habitat and develop a Habitat Conservation Plan.

ACTION:

Explore possibilities of habitat enhancement/protection through working with private landowners (Partners in Fish and Wildlife, other incentive programmes, and landowner agreements, e.g., safe harbour).

1.33 Enhance breeding areas

ACTION:

Develop dune stabilization plan to protect Guanica breeding pond. Provide informational signage. Involve local schools in planting (CANALS: priority).

ACTION:

Construct additional breeding and release ponds. Survey and prioritize additional sites for pond construction in the Guanica area in case of loss of only known breeding pond. Priority areas are Ensenada, Guaniquilla, and Guanica and then potential sites (i.e. Guyanilla) within historic range.

2 PROPAGATE PR TOADS FOR RE-ESTABLISHMENT IN THE WILD

2.1 Establish new captive colonies and obtain necessary founders for captive program to maintain genetic diversity.

ACTION:

Over 16 North American zoos are currently holding toads and all are prepared for breeding. They provide resources, expertise, and funding for recovery.

ACTION:

Obtain 20 tadpoles/toadlets from Guanica and up to 30 from any Northern population as potential founders for captive breeding and release and to sustain the genetic diversity of the SSP breeding population. There are no northern population toads capable of breeding in captivity.

ACTION:

Provide information to Puerto Rican partners by way of Masterplan meeting on genetics of small populations and role of captive breeding (gene drop; effective population).

2.2 Develop captive breeding programme in Puerto Rico

ACTION:

Toads for breeding in Puerto Rico are to be located at the Mayaguez Zoo (Mayaguez University contact Fernando Bird). Working relationship to be encouraged between SSP zoos outside Puerto Rico (e.g., Toronto Zoo) and Mayaguez Zoo (Juan Rivero Zoo)

ACTION:

Provide training in husbandry, breeding and veterinary care. Provide life support systems for holding and breeding toads in Puerto Rico.

ACTION:

Explore possibility of having breeding facilities set up at other institutions (e.g., UPR Humacao, UPR Rio Piedras) if warranted.

ACTION:

Establish a Scientific Advisory Group.

2.3 Captive propagation research

ACTION:

Toronto Zoo has approved two projects that will examine over a two year period nutrition of tadpole and toadlet diets, influence of temperature on development rates, and haematology of toads.

ACTION:

The AZA SSP Coordinator at Toronto Zoo oversees yearly revisions to the husbandry manual.

2.4 Maintain integrity of broodstock.

ACTION:

The AZA Puerto Rican crested toad Studbook is maintained at Toronto Zoo.

3. ESTABLISH THREE NORTHERN AND THREE SOUTHERN POPULATIONS

3.2 Identify reintroduction sites

ACTION:

Survey and prioritize release sites in the Ensenada, Guaniquilla, and Guanica Forest areas. Design and construct release ponds. Initially 5 ponds will be constructed for release or translocation of tadpoles. Determine criteria for suitable release sites.

3.3. Implement and evaluate release program

ACTION:

Make recommendations for breeding at all zoos holding toads with tadpoles returning to the wild for release.

ACTION:

Consider merit of re-introduction to other areas in historic range

ACTION:

Maintain permit flexibility to allow for adaptive management (Ongoing with Atlanta)

4. DEVELOP AN ISLANDWIDE EDUCATION PROGRAMME.

NOTE: These are to be joint AZA and FWS programmes.

4.2 Prepare slide presentations

ACTION:

Develop slide programme for use in schools and at community functions. Develop commentary for use by educators for self guided lessons or with curriculum guide. Slides to include: life cycle of toad, current breeding habitat and importance of community support, radio-tracking research, and role students can play in protecting endangered species.

4.3 Prepare illustrative brochure

ACTION:

Prepare brochure to be widely distributed with natural history information on the toad, how to identify it (compared with *b. marinus*) and soliciting information on new populations.

4.4 Continue to distribute Toronto Zoo poster

ACTION:

New posters will be developed specifically addressing survival in A) the north and B) the south. Poster will highlight contribution made by AZA and 16 institutions supporting the SSP.

IN ADDITION:

Support education objectives

ACTION:

Provide life support and live toads for use in education programming at Quebradillas high school (Ernesto Estremera); Guanica Forest (Miguel Canals); and USFWS Field Office (Ken Foote/Susan Silander)

ACTION:

Develop, test and distribute a curriculum guide for use in grade 5 and 6 primary school science classes. The curriculum guide will highlight the crested toad, endangered species and freshwater ecosystems in Puerto Rico. Consult FWS, educators and education agencies during development of guide. NOTE: THIS ACTION WAS REPLACED WITH A EDUCATIONAL STORY

BOOK

ACTION:

Produce video of toad habitats, breeding, and radio-tracking for use in schools and at interpretive centers.

ACTION:

Develop graphics on toad life cycle (compare with direct development of the coqui); endangered habitats; and story of decline (introduced species; climatic change) for use at USFWS Field Station; Guanica Forest interpretive centre; Mayaguez Zoo; and the Science Park.

ACTION:

Provide lectures to schools within historic range of toads and within 5km of highway 2 as part of education program and to solicit information on new toad locations.

SCHOOLS in NORTHERN towns in order of priority:

Quebradillas, Isabela, Aguadilla, Arecibo, Barceloneta, Dorado and schools between towns.

SCHOOLS in SOUTHERN towns in order of priority:

Guanica, Ensenada, Guyanilla, Ponce, Salinas, Santa Isabel, Coamo and schools between towns.

ACTION:

Produce sapo concho bumper stickers and buttons (COMPLETED IN 1998).

ACTION:

Document accurate chronology of toad re-discovery, wild breedings and captive breedings and releases.

ACTION:

Develop and implement fundraising strategy to support ongoing programming and enlist aid of relevant NGO's.

ACTION:

Develop educational programs with Mayaguez Zoo and other institutions in Puerto Rico.

Remaining recovery objectives:

1.1 Monitor existing populations

ACTION: Explore MOU with DNER for monitoring in Guanica or Quebradillas

1.4 Determine predation and competition by other species

3.1 Classify physiologic features of northern and southern breeding habitat.

ACTION: Obtain data from DNER from previous studies and establish goals for further research.

4.1 Establish a cooperative public information programme with local media (e.g. Puerto Rico Public T.V.).

INVESTIGATE:

1. Memorandum of Understanding (MOU) between USFWS, DNR, and AZA
2. Returning ownership of all toads to PR DNR
3. Joint Coordinatorship for SSP with AZA and USFWS

Puerto Rican Crested Toad Conservation Meeting 2001

The next important planning meeting was the Puerto Rican crested toad conservation meeting hosted by USFWS and AZA crested toad SSP that took place in USFWS Caribbean Field Office in 2001. Due to the importance of Juan Rivero Zoo as an education, display, and breeding facility it was agreed that a follow-up meeting would be scheduled with Juan Rivero Zoo director (see separate minutes for results of meeting held December 6, 2001 at Juan Rivero Zoo).

Meeting began with a review of accomplishments since last meeting in 1998 and a number of 1998 priorities that had been completed as detailed in the USFWS Recovery Plan. There was recognition of the number of students and new people attending the meeting and the increasing transfer of expertise and leadership to new Puerto Rican participants.

- **Habitat analysis** (Jaime Matos/Fernando Bird, UPR Mayaguez)
- **Disease Screening Health Survey of wild toads** (Luis Padilla, St. Louis Zoo). Need to obtain permits now, Miguel Canals has agreed to the need for health screening.
- **Genetic Analysis for Recovery of the crested toad** (Paul Wilson/Kaela Beauclerc, Trent University Forensic Science)
- **Wetland restoration techniques for a threatened species** (Gregory Morris/Elsie Parrilla-Castellar; Miguel Canals/Bob Johnson)
- **Wetland restoration project review** (Gregory L. Morris and associates)
- **Additional research topics** suggested includes: prey and diet; impacts of predation (birds, crabs, odonata larvae and diving beetles, lizards, ants) and introduced species (marine toad, mongoose, rats); impact of pollutants, use of substrates and water for pond imprinting; and evaluation of education, outreach and awareness programs)

Bob Johnson will continue to fundraise on behalf of the SSP and USFWS to provide support for priority projects. USFWS and DNER both agreed that they could provide funds if detailed proposals were submitted.

APPENDIX B:

Puerto Rican Crested Toad Meeting: 2001 Priorities (72 items)

Priority A (55 items)

- ***Pre-apply for DNER and USF&WS permits and sub-permits for research and field collection. (Jaime Matos field research in Guanica forest; Radio tracking in Guanica forest; Health screening in Guanica forest; Collection of northern toads or tadpoles).***

Support Field research:

- ***Jaime Matos field research in Guanica forest***
- Radio tracking in Guanica forest
- Health screening in Guanica forest
- Northern and southern population markers and genetic analysis
- Wetland creation project
- **Determine status in Quebradillas**
- Develop plan for systematic searches for additional toad populations (particularly in the north; use former students from Quebradillas)
 - Northern school contacts in order of priority; Quebradillas, Isabella, Agaudilla Arecibo, Barceloneta, Dorato and schools between towns
- Determine end point for conclusion of searches for northern toads
- Form a sub-group to determine release location for captive bred northern toad tadpoles
- Private landowner partnerships (determine names and numbers)
- Possibility of building ponds under section 6 DNER
- Determine suitability of Cambalache and Guajataca as release sites
- Habitat Restoration in Guanica (create translocation ponds; restore beach sand dunes; restore former breeding sites lost to increased salinity)
- Continue searches for new release and translocation sites in Guanica and along southern coast
- Confirm population trend in Guanica (M Canals data suggests decline from 300 to 50)
- Continue Tadpole translocation (move 1% (minimum of 500 i.e. 1% of progeny from 10 breeding pairs assuming 5,000 eggs laid) of tadpoles at each breeding event to translocation ponds at each breeding event: remove entire population of tadpoles to translocation ponds if threatened by desiccation.
- Develop plan for catastrophic loss (i.e. hurricane, wetland contamination, disease)
- Field research on toad ecology, habitat use, population structure, and restoration.
- Conduct radio telemetry study of toads
- Publish 1992 Moreno/Garcia Principal Component study at Tamarindo (J Matos willing to analyse data)
- Research on health status and disease incidence in wild population
- Research on sources and impact of predation
- Research on impact of pollutants particularly at Tamarindo

- **Education:**
- Develop newsletter and list serve as a recovery communication tool
- Complete education plan for north and south populations. Evaluate effectiveness.
- Develop teacher training workshops for north and south
- Develop web site.
- Develop new toad natural history and conservation video by consolidating existing videos.
- Develop public awareness programme to increase exposure: press releases, activities that will be covered by national news agencies, zoo events, and local hero days.
- Outreach: Develop toad festival (Sapo Concho Day). Use Cuban Croc festival as an example
- Provide more models (marine and PRCT) for out reach
- Develop and distribute coloured identification fact sheets with life size illustrations
- Provide reprints of toad stickers and bumper stickers.
- Provide reprints of toad activity book
- Develop book covers, folder/kit and book marks
- Develop education steering committee.
- Provide lectures to schools within the historic range of toads and within 5km of HW2 as part of an education programme and to solicit information on new toad locations
 - Northern schools in order of priority; Quebradillas, Isabella, Agaudilla Arecibo, Barceloneta, Dorato and schools between towns
 - Southern Schools in order of priority: Guanica, Ensenada, Guyanilla, Ponce, Salinas, Santa Isabel, Coamao, and schools between towns
- Support development of toad exhibit at Juan Rivero Zoo (based on notes from follow-up meeting with Juan Rivero Zoo Director, Marisell Mora Donzalez, and Fred V. Soltero, Veterinarian, and 6 SSP participants at Juan Rivero Zoo Dec.6, 2001)
- Juan Rivero Zoo a centre for:
 - Education (large number of visitors from urban areas; centred between northern and southern populations)
 - Display (adopted by SSP and 22 zoos)
 - Training
 - Marketing
 - Exhibit design
 - Breeding for release
 - Support backup for toads held at UPR Mayaguez
 - Explore Zoo Conservation Outreach Group (ZCOG) assistance
 - Share zoo exhibit connection with the wild (i.e. Guanica Forest)
 - Develop opportunities for Ernesto Estremera students
 - Trained
 - Systematic searches
 - Volunteer educators
 - SSP support
- SSP sponsorship of exhibit (graphics, exhibit design)
- SSP and AZA based staff training

- Develop “local heroes” day to celebrate local conservation efforts
- Develop protocol for care and breeding (including daily requirements)
- Evaluate outcomes
- Continue to bred toads at UPR Mayaguez and develop breeding group at Juan Rivero Zoo
- Transfer single toad at UPR Rio Piedras to UPR Mayaguez
- Investigate Section 6 funding (10-15K for land acquisition and toad research and recovery)
- Determine sources of funding to support recovery
- Student based projects: Develop proposals (i.e. USF&WS) to assist recovery projects (i.e. student based field surveys)
- Student based projects: Explore associating with UPR Aguadilla (over 18 with qualifications). Consider stipend for student based intensive surveys
- Student based projects: Explore volunteer opportunities at Juan Rivero Zoo

Priority B (10 items)

- Juan Rivero Zoo: develop criteria for choosing natural reproduction or hormone induced reproduction
- Provide opportunities for student involvement in recovery activities (field trips)
- Develop educator conservation suitcase
- Develop and distribute small size toad conservation posters
- Investigate potential of toad conservation advertising on products (pop cans)
- Explore potential of using Puerto Rican pond substrate material in captive bred tadpole rearing: research and evaluate if an effective imprinting tool
- Transfer additional genetic lines to Juan Rivero Zoo/UPR Mayaguez for breeding (Transfer 0.0.11 toads from Sedgewick County zoo and remaining 0.1 toad from UPR Rio Pedras to Juan Rivero Zoo/UPR Mayaguez)
- Research on diet and prey species of toads (stomach contents)
- Provide northern toad land owner information and explore potential of DNER/USF&W service partnership purchase of small parcels
- Explore partnership with DNER and USF&WS for potential protection and restoration of Guaniquilla wetlands.

Priority C (7 items)

- Habitat Protection in Quebradillas (**Priority A** if toads found)
- Juan Rivero Zoo: Monitor relationship between barometric pressure and toad reproduction
- Research project: Use of barometric pressure to induce reproduction.
- Captive population in SSP: Increase number of potential founders from 20 to 30 to compensate for less robust potential founders.
- Investigate sponsorship at Juan Rivero Zoo
- Develop toad costume/mask/hat for outreach events

- Develop proposal for sociology based study of the development and effectiveness of a zoo recovery partnership in Puerto Rico

Following this meeting, with support from ZCOG, Biologists from Juan Rivero Zoo participated in husbandry training at US zoos. Two US zoos Audubon and central Florida held toads outdoors to provide background information for rearing in outdoor pools in Puerto Rico during natural weather fluctuations. During this time in Puerto Rico, two large release ponds (4000 and 1000 sq. ft.) were constructed at Manglillo; and new gates and conservation signage were installed at Tamarindo.

Due to the importance of Juan Rivero Zoo as an education, display, and breeding facility, agreed that a follow-up meeting would be scheduled with Juan Rivero director. Priority actions resulting from this meeting are included in priority lists (Appendix B). This was an important benchmark for the SSP in terms of ensuring in situ Puerto Rican driven conservation education programming; having toads visible to Puerto Ricans and island visitors; and as a location for in country captive breeding.

As SSP Coordinator for over 20 years I have many to thank. First the toad itself for giving many challenges and much joy, Puerto Rico and Puerto Ricans are enchanted indeed and I always feel at home here. Miguel Canals and Ernesto Estremera are an inspiration and the toad would not still be part of Puerto Rico's natural heritage without their dedication. I thank Rick Paine, formerly of Buffalo Zoo; Jose Vivaldi, Hilda Diaz-Soltero, Jorge Moreno, Miguel Garcia, Fernando Bird, Enrique Hernandez Prieto, Fred Soltero, Luis Figueroa, Susan Silander, Jim Oland, Gloria Lee, Susan Silander, Carlos Diaz, Silmarie Padron, Marisell Mora Gonzalez, Carlos Pacheco-Matos, Abel Vale, Marelisa Rivera, Norma Villarrubia, Oscar Vazquez, Jose Cuevas, Bob Lacy, Jorge Ortiz, Andrew Lentini, Toronto Zoo for so many years of support, and the AZA Conservation Endowment fund and 22 Zoos and Aquaria that support the crested toad SSP.

Summary of the Recovery Plan for the Puerto Rican Crested Toad (*Peltophryne lemur*)

Silmarie Padrón, USFWS

The Mission of the U.S. Fish & Wildlife Service is to work with others to conserve, protect and enhance fish, wildlife, and plants and their habitats for the continuing benefit of the American people.

The Puerto Rican crested toad was determined to be a threatened species on August 4, 1987, pursuant to the Endangered Species Act of 1973, as amended. Regulations prohibit certain activities involving endangered or threatened species, as specified in Section 9 of the Act, unless permits are issued. This species is the only native bufonid of Puerto Rico and the Virgin Islands. Two populations are known in Puerto Rico (Guanica and Quebradillas). Historically, the species was found in nine locations in Puerto Rico and in one location in Virgin Gorda, British Virgin Islands. Reason for listing this species are the use of already limited breeding sites, vulnerability to habitat destruction and human interference, over utilization, predation and other threats.

The Regional Office in Atlanta approved the recovery plan for the PR crested toad on August 7, 1992. The recovery plan is composed of current status, habitat requirements and limiting factors, recovery objectives and criteria, actions needed, total estimated cost of recovery, date of recovery and implementation schedule. The objective of the PR crested toad recovery plan is to provide directions for restoring the species to a self-sustaining status, thereby permitting it to eventually be removed from the list. Delisting will be considered when at least three wild populations (1,500-2,000 animals) are established and maintained for each race, northern and southern, for ten years and at least five captive populations (300 animals each) have been established.

Actions needed for the recovery of this species are: prevent further population decline and habitat loss, continue to propagate PR crested toads suitable for reestablishment in the wild, establish at least three populations in the north and three in the south and develop an island wide education program. A recovery date will be established once recovery criteria are met.

Implementation Schedule

Priority I

- Monitor existing populations
- Search for historical and new populations
- Manage northern and southern populations
- Protect commonwealth-owned habitat
- Protect existing habitat on private land

Priority II

- Develop habitat conservation plans
- Determine the extent of predation and competition by other species

- Complete establishment of and maintain captive populations
- Develop captive propagation program in Puerto Rico
- Continue or initiate captive propagation research
- Maintain integrity of brood-stock through implementation of breeding program with the American Zoo and Aquarium Association (AZA)
- Classify physiographic features of northern and southern habitat
- Identify introduction sites
- Implement and evaluate release programs

Priority III

- Enhance breeding areas
- Establish cooperative public information programs with local media
- Prepare slide presentations
- Prepare illustrative brochure
- Continue to distribute poster prepared by Metro Toronto Zoo

Other Actions

- Section 7 Consultation
 - Formal and informal consultation with federal agencies
 - Identify possible habitat for the species
 - Establish protocol for searches
- Partners for Fish and Wildlife Program
 - Identify habitat in northern and southern private land
 - Create agreements with landowners to restore possible habitat for the Puerto Rican crested toad

Funding could be possible through Endangered Species funding (Recovery Program), Flex Funds, Partners for Fish and Wildlife Program and National Fish and Wildlife Foundation. This project is possible through partnerships between AZA, states agencies, universities, local schools, and communities.

Habitat characterization for the Puerto Rican Crested Toad (*Peltophryne [Bufo] lemur*) at the Guánica State Forest

Jaime J. Matos-Torres, University of Puerto Rico/Mayagüez

Note: These are the preliminary results of the research currently undergoing. Some results and analyses are not presented.

Introduction

Although amphibians represent the oldest group of terrestrial tetrapods (Pough et al., 2001), actually they are among the most vulnerable vertebrates on earth. Since the end of the 1980s scientists have observed a worldwide decline of amphibian populations. Two examples of these declines include the Golden Toad (*Bufo perezianus*) of Costa Rica, extinct since 1989, and the Mouth-Breeder Frog (*Rheobatrachus silus*) from Australia, which disappeared in 1981. These cases have led to a hypothesis affecting amphibians around the world. Called the “Monteverde Syndrome” (Mattoon, 2000), this hypothesis states factors which could account for amphibian declines. Those factors include: water pollution, which is associated with damage to the eggs, tadpoles and malformations in adults; UV radiation, which affects the organisms at the molecular level; and climatic changes, which interfere with the reproductive cycles and water availability for the organisms. In addition, infectious diseases unique to amphibian populations have been found. Chytrid fungi, saprogenic bacteria and iridoviruses are some of the most currently studied. Finally, habitat loss and destruction is the most widespread factor affecting amphibians. Habitat modifications such as deforestation, wetlands drainage and human development (highways, dams, urban sprawl and expansion of agricultural lands) may be the direct cause of death for organisms that cannot cope with such changes.

In Puerto Rico, habitat loss has been associated with the decline of several amphibian species. One of those species is the Puerto Rican crested toad (*Peltophryne lemur*, Cope 1868), the only endemic toad of Puerto Rico. The individuals of this species are mid-sized (64-120 mm, SVL); characterized by supraorbital crests and a long upturned snout. Although this species historically has been reported in the Virgin Islands and different locations in Puerto Rico, as of today the only known reproductively active population is found at Guánica State Forest, at the southwestern part of Puerto Rico. No toads have been seen in other historically known areas since 1991 (Johnson, personal comm.). In Guánica, this species lives in a low elevation, semi-arid area, with rocky outcrops of limestone that provide shelter for the species. Because of the xeric conditions of the area, this species only breeds when seasonal rains form temporary ponds. Population decline and habitat loss are the major factors that have led this species to be listed as Threatened in 1987 (FWS, 1992).

Actions that could be implemented for the recovery of this species include the identification of habitat and microhabitat preferences. This information can be used to make better decisions on which habitats need to be preserved as well to identify suitable areas for reintroduction. The purpose of this project is to measure the characteristics that encompass the Puerto Rican crested toad's actual habitat. With this information management and recovery programs could be improved.

Objectives

- Identify areas inside the Guánica State Forest in which activity of the toads could be observed.
- Measure environmental and ecological variables in the identified areas.
- Describe those variables.
- Use the obtained information in future evaluations for conservation and management of the species.

Methods

Night searches were made at the Guánica State Forest looking for *Peltophryne* activity. All toads observed were captured and basic measurements were taken (snout-to-vent length/minimum head width) (Fellers et al, 1994), the site was marked and identified with a global positioning system (GPS) (Hayek et al, 1994). At each site a 10m by 10m quadrat was established. At each quadrat, 25 samples of 1m by 1m were systematically taken. In each 1m by 1m sample, a description of vegetation type, composition and frequency was made. The description at each sample also included surface composition, canopy cover and horizontal obscurity. To correct for habitat selection bias each 10m by 10m section was paired to other equally sized quadrats placed randomly at 20m from the first.

The identification of the vegetation association type was made according to Dugger (1979), who describes nine vegetation associations for the Guánica Forest based on the density and composition of species. Vegetation association types were scored as rocks and cacti, scrubland, caducifolious forest and evergreen forest.

To estimate the vegetation composition at each sample, only plants with stems above knee-high (AKH) were considered. The proportion of all AKH plants classified as arboreus, cacti, weedy, succulent or other was calculated. The frequency of all trees AKH was calculated by species.

The surface composition was described as a proportion of leaf litter, rocks, vegetation, crevices, and logs. The canopy cover was estimated using an ocular tube by which a proportion of the tree crown projection onto the ground surface was measured. The horizontal obscurity is the visual obstruction caused by vegetation at the horizontal plane. The horizontal obscurity was estimated using a Nudds table (Higgins et al, 1996) by which visual obstruction of the vegetation can be assessed at 0.5m intervals above the ground.

All the information gathered was entered into a geographic information system (GIS) for predicting and modeling the areas more suitable as habitat for the crested toad (Higgins et al, 1996).

A description of the toads' breeding areas also was made. The measured characteristics of the breeding areas were temperature, pH, conductivity and salinity.

Results

A total of 24 plots were established related to four vegetation associations: rocks and cacti, scrubland, caducifoleus and evergreen forest. The rocks and cacti association encompassed 46% of the studied sites and contained 58% of all the toads observed. All toads observed in the rocks and cacti were males. The predominant vegetation type in the rocks & cacti was arboreus (67%) which included bushes and trees like: *Thouinia portorricensis*, *Exosterma caribaeum*, *Bursera simaruba*, *Croton lucidus*, *Croton discolor*, *Reynosa uncinata*, *Comocladea dodonea*, *Melochia pyramidale*, *Krameria ixina* and *Plumeria alba*. The second most predominant vegetation type in the rocks and cacti association was xerophytic (25%) with two species: *Cephaloceres royenii* and *Melocactus intortus*. The other types of vegetation consisted of herbaceous plants (3%) and weeds (5%). The most predominant surface characteristic in the rocks and cacti association was bare rock (52%), followed by leaf litter (36%). Other surface characteristics found on the rocks and cacti were green vegetation (6%), dirt (3%), crevices (2%) and logs (1%). The average temperature found in the rocks and cacti was 27.72°C with an average relative humidity of 66.63%.

The scrubland vegetation association included 29% of the studied sites and contained 17% of the toad observations. All toads observed in the scrubland vegetation were males. Arboreous was the predominant vegetation (58%) and contained trees and bushes like: *Bucida buceras*, *Leucaena leucocephala*, *Pithecelobium inguis*, *Euphorbia petiolaris*, *Securinega acidoton*, *Schaefferia frutescens*, *Guaiacum officinale*, *Bourreria virgata*, *Eugenia rhembea*, *Capparis indica*, *Capparis flexuosa* and *Erythroxylum aureolatum*. The second vegetation type was xerophytic (12%) which contained two species: *Opuntia monliniformes* and *Cephaloceres royenii*. The weedy vegetation type scored 24%. In the scrubland there are succulent plants that compose 6% of the vegetation with the genus *Sansiveria* predominant. The surface characteristic predominant in the scrubland is leaf litter (67%). The green vegetation at surface level composed 13%. Dirt composed 11% with logs and rocks composing 5% and 4% respectively. The average temperature for the scrubland was 25.93°C with an average relative humidity of 81.17%.

The caducifoleous association included 17% of the sites and contained 17% of toad observations. Two of the four females found in the study were observed in the caducifoleous association. Arboreus encompasses 44% of the vegetation and included trees and bushes like: *Gymnanthes lucida*, *Erythroxylum aureolatum*, *Ziziphus reticulata*, *Pisonia albida*, *Capparis flexuosa*, *Sideroxylon fruticosum*, *Adelia ricinella*, *Lasiacis divaricata*, *Amyris elemifera* and *Prosopis indica*. Succulent plants form 26% of the vegetation with *Sansiveria* predominant. Weedy vegetation comprises 23% of the proportion and xerophytic vegetation 6% with species like *Cephaloceres royenii* and *Opuntia moliniformes*. The predominant surface characteristic at the caducifoleous association was leaf litter (51%). Other important surface characteristics were green vegetation at surface level (28%), rocks (16%), logs (35%) and dirt (2%). The average temperature found at the caducifoleous association was 27.87°C with an average relative humidity of 76.32%.

The evergreen association includes 8% of the sites. Eight percent of the toads were observed at the evergreen association including two other females. The vegetation types composing the evergreen association were arboreous (75%), weeds (22%), xerophytes (2%) and succulents

(1%). The predominant tree and bush species for the evergreen association were: *Prosopis indica*, *Eugenia rhembea*, *Capparis indica*, *Capparis flexuosa*, *Eugenia rhembea*, *Capparis flexuosa*, *Erythroxylum aureolatum*, *Pithecellobium inguis*, *Leucaena leucocephala*, *Bucida buceras*, *Pictetia aculeate*, *Krugiodendron ferrum* and *Myrciantes fragans*. The surface characteristics of the evergreen association consisted of only three components: leaf litter (82%), rocks (12%) and green vegetation (6%). The average temperature found at the evergreen association was 27.09°C with an average relative humidity of 83.11%.

The breeding areas for the crested toad consist of the ponds of Tamarindo, Atolladora and Aroma. The Tamarindo pond is actually the preferred area for toad breeding. It is located at the end of the PR 333 road with coordinates 17°57.260 N and 066°50.903 W. Due to the geographical characteristics of the Tamarindo pond, it could be divided in three: Tamarindo north, Tamarindo center, and Tamarindo south. The north and center sections of the Tamarindo pond seem to be the preferred by the crested toads for breeding. The average pH for the north section was 7.783. The average conductivity was 15.842 mq/cm. The average temperature was 30.53°C with an average salinity of 0.97%. As for the center section of the Tamarindo pond its pH averaged 7.796, with a conductivity of 5.207 mq/cm, a temperature of 29.53°C and a salinity of 0.269%.

The Aroma pond is located at the coordinates 17°57.284 N and 066°51.048 W. Although historically this pond was used for breeding, no egg laying was observed during this investigation. The Aroma pond has an average pH of 7.306. The average conductivity was 34.1 mq/cm. The temperature averaged 33.12°C. The salinity at Aroma was of 2.16%. The Atolladora pond is located at the coordinates 17°57.423 N and 066°51.251 W, and like the Aroma pond this area was historically used by the crested toads, but no reproduction was accounted during this investigation. The pH at Atolladora was 7.59. The conductivity was 26.8 mq/cm. The temperature 32.55°C and the salinity 1.57%.

The results of the characteristics for the breeding ponds shows that Tamarindo north has a significantly higher pH than Atolladora or Aroma ($p < 0.00$, $df=13$, $\alpha = 0.05$), but not significantly different from Tamarindo center ($p=0.859$, $df=10$, $\alpha = 0.05$). Temperature values were not significantly different from other ponds. In terms of salinity Tamarindo north was significantly lower than Atolladora ($p < 0.003$, $df=13$, $\alpha=0.05$) and Aroma ($p < 0.000$, $df=17$, $\alpha=0.05$), and significantly higher than Tamarindo center ($p < 0.000$, $df=9$, $\alpha=0.05$).

Using GIS systems and tools efforts were made to predict the distribution of the crested toads through the studied habitat. A supervised classification of satellite images shows four vegetation types based on the Near Infrared Vegetation Index (NIVI). The vegetation types were converted to distance images and used as a variable in a logistic regression model obtaining a partial regression coefficient of 0.089. Using Digital Elevation Models (DEM), areas were identified where rainfall runoff water distributes in the forest. The runoff water image was also entered into the regression model, with a partial regression coefficient of 0.0056. The last variable used in the model was the distance from the breeding ponds. This variable yielded a partial coefficient of 0.004. All these variables were correlated to the sites in which toads were observed and GPS points were taken. The regression coefficient (R^2) obtained for the correlation was 0.38. The

probability image obtained by the GIS analysis showed a higher probability of finding toads in the forested areas to the north of the breeding areas.

Discussion

The crested toad was found in all vegetation association types. Although a higher proportion of toads were found in the rocks and cacti, this could be directly correlated to the wide distribution of this vegetation association in the studied areas. The rocks and cacti areas have a lower average humidity and the vegetation was scarce which could indicate a low shelter profile, but as surface characteristics indicate these areas has the higher percent of cavities that are reported to be used by the toads as shelter. Finding the only females at the caducifoleus and evergreen forest, which present a higher humidity and more vegetation cover, may be a signal that females wander less and look for better sheltered areas in which they could spend more time in the dry season. As of for the breeding areas, the ponds show significant differences in pH and salinity. These differences could be critical for breeding site selection and offspring survival. GIS analysis shows a higher probability of finding toads in the forest areas around breeding ponds. The model shows a high fitness in explaining the variability of the data, making it an useful tool for future predictions.

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Historia Natural del Sapo Concho en Quebradillas

Ernesto R. Estremera, USFWS

La Historia Natural del Sapo Concho en Quebradillas comienza en el año 1974 cuando se descubre la especie en el barrio Cocos de nuestro municipio. Dicha especie no había sido descubierta en Puerto Rico desde el año 1966 cuando el Dr. Julio García Díaz la encuentra en el Barrio Cotto del municipio de Isabela.

Tan pronto fue descubierta en Quebradillas (1974) me dediqué con mis estudiantes y especialistas en el estudio de los anfibios a visitar los lugares donde fue descubierta la especie. Se encontraron decenas de especímenes que fueron llevados a diferentes centros de estudio tales como: Colegio de Mayagüez, Zoológico de Mayagüez, Cayey y la Estación experimental de Isabela.

La noticia de este descubrimiento trajo a Quebradillas numerosos científicos entre ellos al Sr. Bob Johnson cuyo interés era el llevar la especie a Canadá (Zoológico de Toronto) para reproducirla en sus facilidades y luego introducir la misma a su hábitat natural nuevamente.

Todos mis estudiantes y compañeros maestros nos sentimos muy felices porque nuestro sapo concho pronto aumentaría su población en nuestro pueblo y podríamos estudiar la especie en su estado natural. La felicidad no duro mucho tiempo, pues en visitas que realizamos años tras años en los lugares donde se encontraba la especie, iba disminuyendo la cantidad de individuos hasta que en el 1992 sólo pudimos encontrar un ejemplar juvenil y desde entonces no hemos encontrado ejemplar alguno.

Aunque llevamos once (11) años sin volver a ver nuestro sapo concho, no hemos dejado de llevar conferencias sobre la vida natural del Sapo Concho Puertorriqueño a la población del noroeste de Puerto Rico. Hemos llevado conferencias a la UPR- de Aguadilla, la Universidad Interamericana de Aguadilla, Escuelas Públicas de Camuy, grupos de niños de la Iglesia Carismática de Quebradillas, Escuelas Superiores de Quebradillas, Asamblea Municipal de Quebradillas, Universidad Metropolitana, en fin hemos educado a cientos de puertorriqueños sobre la importancia de conservar y proteger los hábitat donde el Sapo Concho se encuentra.

Solo me restan tres años para retirarme como maestro de ciencia y desearía, como desean mis estudiantes y mis compañeros maestros, que nuestro sapo concho no desaparezca para siempre de nuestros hábitat naturales, que traten de que nuevamente se pueda oír el canto de tan maravilloso organismo en nuestro pueblo.

De nuestra población han surgido millones de renacuajos y de ellos cientos de adultos que han sido llevados a numerosos zoológicos a través del mundo.

Han viajado mucho, ya es hora que vuelvan a Quebradillas, a su hábitat natural, a la casa a la que pertenecen. Se que todos unidos podemos contribuir a que Quebradillas vuelva a tener la población que dio origen a que hoy estemos aquí reunidos.

Puerto Rican Crested Toad Captive Population

Andrew Lentini, Toronto Zoo

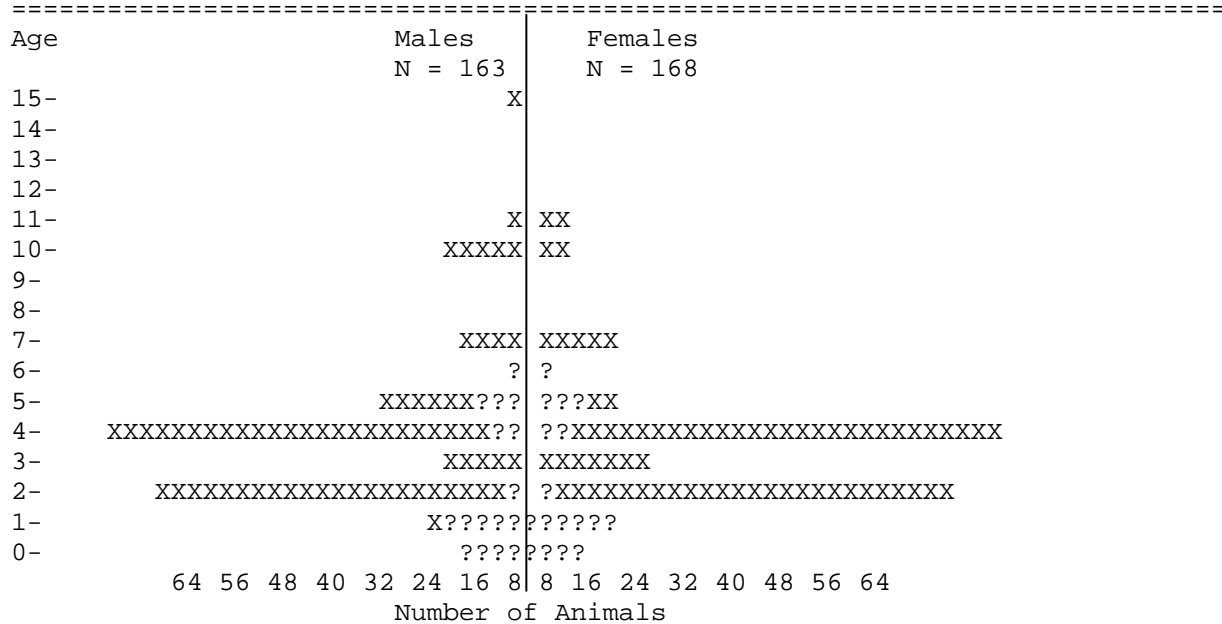
As of 2004.01.16 the captive population consisted of 131.136.63 Southern population animals (330) at 20 institutions, and 2.6 Northern toads at Toronto Zoo.

Southern Population

There are 15 wild-caught toads still alive within the captive population. Seven are founders and eight are potential founders (not yet having reproduced). The age pyramid shows a good distribution with the majority of animals being under five years of age. The mean kinship for the population is 0.052 and a current gene diversity of 0.9480.

Age Pyramid Report Southern Population

Status: Living on 16 Jan 2004



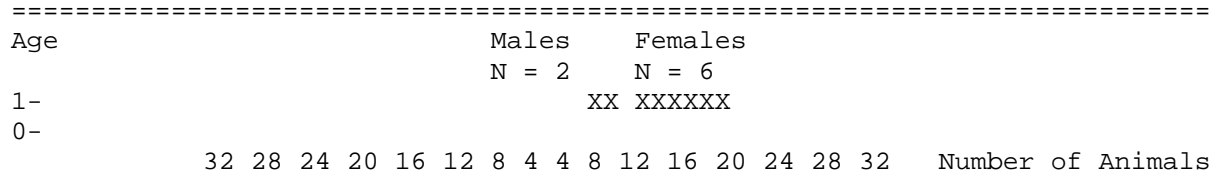
X = Specimens of known sex. ? = Specimens of unknown sex.

Northern Population

There are no wild-caught founders from northern population still living in captivity. All eight living toads are siblings from a March 2002 breeding. This population has a mean kinship of 0.4648 and future breeding would result in a high inbreeding coefficient.

Age Pyramid Report Northern Population

Status: Living on 16 Jan 2004



Genetic Structure of Puerto Rican Crested Toad Populations Applications to Recovery

Kaela Beauclerc, Trent University

Conservation Concerns

Historically nine populations; only two remain

- Quebradillas (North): only a few captive animals remaining
- Guanica (South): only extant wild population

Northern and southern populations are separated by potential biogeographic barrier (interior)

- main question is: have these populations been evolving independently for some time and become cryptic species, or is isolation recent due to habitat loss?

Species status of these populations is critical for proper conservation

- if they are cryptic species, then they must continue to be managed independently
- if separation is recent, then it may be desirable to pool the captive colonies together to increase genetic variation
- also creates potential to release more abundant southern animals into historical northern range

Previous Genetic Studies

Lacy and Foster (1987)

- electrophoretic studies showed moderate levels genetic variation
- divergence of northern and southern populations typical to that of isolated populations or subspecies
- suggested they have been separated for up to 1 million years

Goebel (1996)

- mitochondrial studies showed low levels of genetic variation
- 2 haplotypes; distinguish northern and southern populations
- cytochrome *b*: no variation
- control region: 1.7% sequence divergence
- restriction sites: 2.5% sequence divergence
- indicates that the northern and southern populations are divergent: greater genetic differentiation than between other subspecies or species of *Bufo*
- suggests that northern and southern populations should continue to be conserved distinctly

Objectives

Additional genetic profiling will further evaluate the degree and date of separation of these populations

- determine status as separate species, subspecies, ESUs
- assess the appropriateness of combining captive colonies and/or reintroducing southern animals to northern areas
- assess historic and contemporary levels of genetic variation that exist within and between populations

- determine the levels of gene flow or divergence between populations
- resolve taxonomic issues regarding subspecies/species status
- evaluate the genetic health of the captive breeding colonies

Methods

Specimens

Genetically profile captive breeding colonies

- could suffer from inbreeding or low genetic variation which may affect success of reintroduction
- Obtain museum specimens for extinct populations
- increase representation of northern populations—current sample size is insufficient for rigorous genetic analyses
- allow comprehensive estimate of levels of genetic variation and gene flow
 - compare historical to contemporary levels
 - determine if gene flow existed prior to disturbance
- evaluate whether captive animals genetically represent the historic populations

Sampling

Captive breeding animals

- internal tissues provided post mortem

Toe clips collected from wild individual

Museum samples

- approximately 50-80 specimens
- internal tissues preferred; least damage from formalin fixation

Genetic markers

Mitochondrial DNA sequences

- Control region: highly variable, amenable to intraspecific studies
- Cytochrome *b*: more conserved; useful to study relationships among higher taxa, distantly separated groups

Microsatellite loci

- highly polymorphic tetranucleotide repeats
- fine resolution among closely related groups

Formalin Extraction

Museum samples are formalin-fixed, EtOH-preserved

- formalin creates cross-links within DNA, making it difficult to extract and amplify large fragments

Must develop an effective protocol to extract DNA from formalin-fixed tissues

- wash with glycine buffer prior to extraction to remove excess formalin
- Qiagen vs. phenol-chloroform extractions
- chemical additives to break cross-links (dithiothreitol, DTT or dithioerythritol, DTE) and aid amplification (bovine serum albumin, BSA)
- varying PCR cycling conditions

Results

Formalin extraction

Extracted DNA from formalin-fixed N leopard frogs

- used 36 different treatments

Successfully amplified 400 bp of cytochrome *b*; could not amplify larger fragment

BSA increased amount of amplified product

No consistent relationship between extraction technique and amplified product

- depends primarily on fragment size Sequences matched control

“TouchDown” PCR protocol improved product yield

- annealing temperature ramps down; increases number of cycles

Tested on true museum specimens of Blanchard’s cricket frog

- 300 bp of cytochrome *b* gene
- no amplification; only unusable smearing

Museum samples are older/more degraded than leopard frog samples

Try a new approach: Formalin removal from archival tissue by critical point drying (Fang et al. 2000; Biotechniques)

- successively wash samples in increasing ethanol (30%-100%)
- dry for 2 hours in Critical Point Dryer with liquid CO₂

Claims to remove formalin and reverse cross-linking-performed on fish, amphibian, reptile, bird, and mammal tissue that had been fixed for 16-70 years

- extracted DNA was virtually identical to control DNA: HMW (> 20 kb), yields ~100 micrograms
- amplified 400 and 1200 bp mtDNA, 1800 bp nuclear DNA

Microsatellite development

Enrichment protocol to develop tetranucleotide microsatellites

- select the regions of DNA containing the repeat *prior* to cloning and screening

Probed for GATA and AAAT repeats

- currently have primers for 11 loci to begin screening for polymorphism

*Cytochrome *b* gene*

Sequenced 500-652 bp of cytochrome *b* gene

- 24 samples: 21 southern and 2 northern animals + one published sequence (GenBank)

No variation; single haplotype in all samples

Control region

Sequenced 1022 bp of control region

- 28 samples: 27 southern and 1 northern animal

Two haplotypes: distinguish north and south

- 15 variable sites; mostly transitions (C-T or G-A)
- 1.5 % sequence divergence

Conclusions

Results of genetic work are similar to those of previous work

- no cytochrome *b* differences
- two control region haplotypes, one in North and one in South
- no sharing of haplotypes between N/S

- N/S divergence based on control region suggests ~750 000 years of separation
Suggests N/S populations are distinct, but to what degree?
Profiling using microsatellites, and incorporation of museum specimens, is needed to clarify relationships

Record of Large Rainfalls and Sapo Breeding Activity

Fecha	Precipitación	Reproducción	Localización	Observaciones
Julio 3-5, 84	9.71" (24.66cm)	Si	Tamarindo, Aroma, Atolladora	Primer evento reproductivo observado por Migue Canals. 800-1000 sapos adultos
Sep 17-21, 84	10.34" (26.26cm)	Si	Tamarindo, Aroma, Atolladora	>100 sapos adultos
Nov 4-5, 84	6.91" (17.55cm)	No	Tamarindo, Aroma, Atolladora	No renacuajos. Solo 7 machos
Oct 5-6, 85	16.15" (41.0cm)	Si	Tamarindo, Aroma, Atolladora	400-500 sapos adultos > hembras, < 100 machos (Relación macho/hembra = 4-5:1)
Oct 23-24, 85	4.68" (11.90cm)	Si	Tamarindo	No censo, sapitos observados en el área (Nov 21, 85)
May 10-13, 86	9.11" (23.14cm)	Si	Tamarindo	No censo, sapitos observados en el área (Junio 5, 85)
Junio 20-21, 87	3.86" (9.80cm)	Si	Tamarindo, Atolladora	7 amplexus. Metamorfosis completada en 15 días
Nov 23-24, 87	4.06" (10.31cm)*	No		
Nov 26-27, 87	7.88" (20.0cm)	Si	Tamarindo	>100 adultos
Ago 24, 88	6.79" (17.25cm)	Si	Tamarindo	>50 machos, 14 hembras; 11 en amplexus
Sep 23-24, 89	3.40" (8.60cm)	Si	Tamarindo	No censo, sapitos observados Oct 22, 89 (Huracán Hugo)
Jun 14, 90	5.85" (14.85cm)	Si	Tamarindo	121 machos, 41 hembras; 18 amplexus (Estudio telemetria)
Jun 15, 90	3.20" (8.12cm)	No		
Oct 18-21, 90	5.90" (14.98cm)	Si	Tamarindo	8 sapitos observados Nov 27, 90
Ene 5, 92	4.38" (11.13cm)	Si	Tamarindo	47 machos, 13 hembras
Abril 21, 92	3.16" (8cm)	No		
May 22-23, 92	5.20" (13.20cm)	Si	Tamarindo	14 amplexus
Nov 20, 93	3.08" (7.82cm)	No		
Sept 19-23, 94	5.10" (12.95cm)	Si	Tamarindo	4 machos. Renacuajos observados 27 Sept 94
Oct 16-17, 94	4.12" (10.46cm)	Si	??????	Sapitos observados en cueva del refugio
Ago 17-18, 95	4.20" (10.66cm)*	No		
Feb 4, 98	3.20" (8.13cm)*	No		
Ago 21-25, 98	3.65" (9.27cm)*	No		
Sept 21-22, 98	5.95" (15.12cm)	Si	Tamarindo, Atolladora, Aroma	Huracán Georges, 179 machos, 37 hembras; 21 amplexus
Oct 21-24, 98	3.63" (9.20cm)	No		
Abril 17-18, 00	3.57" (9.06cm)	No	Tamarindo	2 machos observados
Ago 22-23, 00	3.60" (9.14cm)	Si	Tamarindo	5 machos, 1 hembra; 1 amplexus
Sept 17-27, 00	9.73" (24.70cm)	Si	Tamarindo, Atolladora, Aroma	153 Machos, 34 hembras; 27 hileras de huevos
May 7-10, 01	9.70" (24.63cm)	Si	Tamarindo	31 machos, 4 hembras; 4 amplexus
Ago 22, 01	2.40" (6.10cm)	Si	Tamarindo	13 machos, 1 hembra; 1 amplexus. Desección
Abril 20-22, 02	2.53" (6.43cm)	Si	Tamarindo	31 machos, 9 hembras; 5 amplexus, 5 hileras huevos, translocación

Sept 13-15, 02	4.50'' (11.43cm)	Si	Tamarindo	31 machos, 7 hembras; 5 amplexus, 4 hileras de huevos (Jaime Matos)
Abril 18-20, 03	7.5'' (19.05cm)	Si	Tamarindo, Atolladora, Aroma	66 machos, 12 hembras; 12 amplexus, 14 hileras de huevos
Oct 8-9, 03	2.98'' (7.56cm)	Si	Tamarindo, Atolladora, Aroma	101 machos, 20 hembras, (28 hileras de huevos); 11 machos, 3 hembras (7 hileras de huevos); 4 machos, 4 hembras (4 hileras de huevos)
Oct 8-9, 03	2.98'' (7.56cm)	Si	Tamarindo, Atolladora, Aroma	1 macho, 1 hembra (1 hilera de huevos)
Sept 14-15, 04**	2.65'' (6.73cm)	Si	Tamarindo, Atolladora	212 machos, 18 hembras, 15 hileras huevos-Huracán Jeanne
Nov 10-13, 04**	12.76'' (32.41cm)	?	Tamarindo	6 machos
May 17-18, 05**	6.20'' (15.75cm)	Si	Tamarindo, Atolla, Aroma, Mangillo	538 machos, 20 hembras, 4 amplexus, 14 hileras huevos
July 5-7, 05**	1.96'' (4.98cm)	Si	Tamarindo, Aroma, Atolladora	Depresión Tropical, 120 machos, 10 hembras, 37 amplexus, 53 hileras huevos
July 19-20, 05**	2.6'' (6.65cm)	Si	Tamarindo, Aroma, Atolladora	Depresión Tropical, 23 machos, 20 hembras, 13 hileras huevos
October 7-9, 05**	4.76'' (11.90cm)	Si	Tamarindo, Atolladora, Aroma	Depresión Tropical, 1468 machos, 792 hembras, 345 amplexus, 325 hileras de huevos.

* Reference: Miguel Canals, Unpublished Data

**The last two years (2004 and 2005), pairs in amplexus recorded have already been included in the male/female totals. In all previous years (1984-2003), pairs in amplexus are in addition to male/female totals.

**Population and Habitat
Viability Assessment
For the Puerto Rican
Crested Toad**



**SECTION 5
Working Group Reports**

Priority Issues: PRCT PHVA

General Concerns

Note: Listings below in order of informal voting.

1. Physically secure habitat and manage for needs of toads
2. Life history table
3. Look for new areas in N and S where toads may be found
4. Assure adequate law enforcement to protect toad and habitat
5. Establish protocol to search for toads in North
6. Continue to improve captive propagation
7. Develop local network of government, scientists, NGOs, etc. for toad
8. Identify specific ponds and funding
9. Continue to secure additional founders to maintain genetic health of captive populations
10. Restore other areas in South
11. Determine criteria for quality of release sights
12. Consider Virgin Gorda as possible other release site
13. Should we change the common name of the toad to make it distinctively Puerto Rican?
(Done)

Needs/Concerns

1. Exhaustive survey of wild populations
2. Acquisition of critical habitat
3. Work directly with wild populations
 - a) mark-recapture
 - b) Surveys of numbers, density

4. Increase public awareness, community support and profile in PR
5. Speed up genetic work so we know what we are working with in North and South and identify contacts at museums with samples
6. Predator control/ determine predators
7. Coordinate funding strategies
8. Determine what to do if we do not find more toads in North
9. Determine how (or if) to use “hybrid” concept for sapo concho in North and South: consider other terms – e.g. intergrade
10. In North – is species present?
11. In South – more reproductive sites
12. Need to expand Guanica population
13. Continue and increase protection of natural pond (Guanica)
14. Health assessment of toadlets from releases – compare to wild, compare among ponds
15. Disease screening of south and captive populations
16. Get more NGOs involved
17. Determine how to differentiate between tadpoles of *Peltophryne lemur* and *Bufo marinus*.

Management Working Group Report

Priorities

1. Physically secure habitat, manage, and protect for needs of toads.
2. Establish plan for Northern population.
3. Secure more founders for captive populations.
4. Establish plan for Virgin Gorda population

Southern population

Historic populations at Tamarindo, Aroma, Atolladora

- Secure habitat
- Restore sand dunes to protect from salt water contamination or pond breaching during strong storms: DNER,USFWS
- Law enforcement to prevent inappropriate land use that negatively impacts toad habitat (vandalism of signage, gates, barriers...): DNER, Local Police
- Close parking lot to avoid chemical contamination of breeding pond and damage to habitat by vehicles: DNER, City Of Guanica
- Employ a shuttle service to ferry visitors from parking in town to park.
- New parking area in a less sensitive area of park
 - Close road to parking lot during migration of metamorphs: DNER
- Change use of area: DNER
 - More low impact education use: DNER, USFWS, JR Zoo, AZA
- Designate critical areas as Wildlife use only and prohibit other uses that may negatively impact toads and habitat: DNER
- Emergency action plan in case of disaster that will allow for mitigation: DNER
- Purchase remaining 20 acres of forest that are still privately owned: DNER, NGOs, DOT
- Voluntary
- Expropriation
 - Population management
 - Control predation and competition (determine species: dogs, mongoose, rats, dragonfly, B. marinus): DNER
- Improve translocation ponds: DNER, AZA, USFWS
- Continue releases at levels that will saturate predators and improve survival
- Add drainage
- Enhance existing habitat: USFWS, DNER, AZA
 - Natural pond
 - Edge habitat: reforestation along roads and area to south
 - Corridors for metamorph dispersal

Manglillo

- Secure Habitat

- Law enforcement: DNER, Local Police
 - Prohibit motorized vehicles
- Change use of area to low density educational camping. DNER
 - More education: DNER, USFWS, JR Zoo, AZA
- Wildlife use: DNER
- Emergency action plan: DNER
- Fire prevention program: DNER
- Construct perimeter fence: USFWS, DNER
- Reforestation: USFWS, DNER
- Develop infrastructure: DNER
- Water supply
- Drain pond
 - Population management
 - Mitigation of threats
 - Control predation and competition (determine species: dogs, mongoose, rats, dragonfly, B. marinus): DNER
 - Continue releases: DNER, USFWS, AZA
 - Improve translocation ponds: DNER, USFWS, AZA
 - Open canopy: DNER, AZA
 - Build interpretation hut: DNER, AZA
 - Enhance existing natural pond: USFWS, DNER, AZA
 - Hydrology
 - Edge habitat
 - Corridors

Options and Management Strategies for Northern Toads (may apply to Virgin Gorda)

Research performed in North will answer the question “Is there an extant population? Regardless of whether or not toads are found in North, the following management strategies apply:

- Establish land ownership and explore conservation strategies: DNER, USFWS
- Public-owned land
 - Commonwealth
 - Land Authority
 - DNER
- Private-owned land
 - Initiate landowner contact: USFWS, DNER
 - Options to secure land in private ownership
 - Conservation easement: DNER
 - Partner for Wildlife program: USFWS
 - Safe Harbor Agreement: USFWS
 - Legal issues: introduction of listed species on private land has consequences for land owner
 - Potential sites
 - Arecibo sites are under a conservation agreement in perpetuity

- Cambalache Forest is a government property
 - Citizens of the Karst: Abel Vale
 - NGOs
 - Conservation Trust
 - Toledo family
- Purchase: DNER, NGOs
 - Voluntary
 - Expropriation
- Population management
 - Capture a representative sample (at least 20 individuals) of the existing population for a captive breeding program to assure that a captive population exists as an assurance colony against extinction and to serve as a source population for a rapid reintroduction: DNER, USFWS, AZA
 - Physical protection: USFWS, DNER
 - Perimeter protection of ponds to prevent bicycles and motorized vehicles from damaging natural and restored or created habitat
 - Mitigation of threats: DNER, USFWS
 - Control predation and competition (determine species: dogs, mongoose, rats, dragonfly, B. marinus): DNER
 - Additional pond construction or enhancement to provide habitat for wild toads and captive release program animals: DNER, AZA, USFWS
 - Population augmentation: DNER, AZA, USFWS
 - Potential sites: Cambalache, Arecibo, etc. Habitat assessment and site selection of potential areas will be carried out by DNER and USFWS
 - Given our current knowledge base a decision on a source population of release animals cannot be made until genetic studies are done. Current source population options are:
 - Use only offspring from northern animals currently held in AZA zoos
 - Likely inbred
 - Too few animals and genetic diversity is likely very low
 - Use only offspring produced from breeding of northern animals currently in captivity with southern animals currently in captivity.
 - Likely inbred
 - Too few animals and genetic diversity is likely very low
 - Use only offspring from southern animals currently held in AZA zoos
 - Coming from the south these may be poorly adapted to mesic habitat
 - May be capable of adaptation (climate in north has changed in past and northern toads have already had to adapt, southern toads may therefore still have the original diversity)
 - Introduce offspring produced from northern animals currently held in captivity and introduce offspring produced from breeding of northern animals currently in captivity with southern animals currently in captivity.
 - Provide greater genetic diversity and reduce inbreeding

Potential Founders for captive population

- Collecting tadpoles has the least impact on the wild source population: DNER, USFWS, AZA
 - 20 collected (assuming that 18n will survive)
 - Collect every 4 years to provide added genetic diversity for captive population
- To maximize genetic diversity need to capture unrelated animals. This can be done by collecting from:
 - Different egg masses
 - Different locations of pond
 - Different breeding events of same year since the same female will not breed more than once in a year.

Experimental introduction of captive reared northern animals

The management working group recommends that a release pond be established for captive-bred northern toads to be used until a decision is made on the genetic composition of a source population for northern reintroduction. Habitat assessment and site selection of two potential areas identified by DNER and USFWS are in Arecibo and Cambalache forest. Both areas are secure. Arecibo sites are under a conservation agreement in perpetuity and the Cambalache forest is a government property. A release pond will need to be constructed by DNER and any available volunteers (AZA, NGOs, Estremera students). Estimated costs including material for pond and supporting structures (fencing, plumbing infrastructure, site modification) would be \$5,000. Up to half may come from in-kind contributions. Toronto Zoo plans to breed captive northern toads in late 2003 or early 2004; therefore, a site needs to be ready in that timeframe.

Prioritized List of Management Actions

OBJECTIVE	PRIORITY A, B, C	ORGANIZATION AGENCY	CONTACT PERSON	ESTIMATED COST	TIMELINE
Experimental introduction of captive reared northern animals	A	USFWS, DNER, AZA, VOLUNTEERS	Enrique Hernandez	\$5K	By Feb 2004
Initiate a sustained release program of captive bred animals to north	A	USFWS, DNER, AZA	Bob Johnson	In kind	2004
Secure additional founders for southern populations	A	USFWS, DNER, AZA	Miguel Canals	In kind	Ongoing, every four years
Capture a representative sample (at least 20 individuals) of the existing northern population as a source population for a rapid reintroduction.	A	DNER, USFWS, AZA	Enrique Hernandez	In kind	Ongoing
Continue release of captive bred animals to support Manglillo site	A	USFWS, DNER, AZA	Miguel Canals	In kind	Ongoing, yearly

Restore sand dunes to protect from salt water contamination or pond breaching during strong storms at Tamarindo	B	DNER,USF&WS	Miguel Canals	\$15K	2005
Law enforcement to prevent inappropriate land use that negatively impacts toad habitat (vandalism of signage, gates, barriers...) at all sites	A	DNER, LOCAL POLICE	Miguel Canals	In kind	Ongoing
Close parking lot to avoid chemical contamination of breeding pond and damage to habitat by vehicles at Tamarindo	B	DNER, CITY OF GUANICA	Miguel Canals	In kind	Under study
Change designated use of area at all sites	A	DNER	Miguel Canals	\$5K	2005
Emergency action plan in case of disaster that will allow for mitigation for Guanica sites	A	DNER	Miguel Canals	In kind	2004
Purchase of remaining 20 acres of forest that are still privately owned in Guanica	A	DNER, NGO's, DOT	Miguel Canals	\$600K	2005
Control predation and competition at all sites	B	DNER	Miguel Canals	\$1K	2005
Improve translocation pond at Ballena	B	DNER, AZA, USFWS	Miguel Canals	\$1K	2005
Enhance existing habitat (pond, edge habitat, corridors, reforestation) for Guanica sites	B	USFWS, DNER, AZA	Miguel Canals	\$8K	2005
Fire prevention program for Manglillo	A	DNER	Miguel Canals	In kind	2004
Construct perimeter fence at Manglillo	B	USFWS, DNER	Miguel Canals	\$.5K	2005
Develop infrastructure (Water supply, drain) At release and translocation ponds	A	DNER, USFWS	Miguel Canals	In kind	2004
Open canopy to allow water temperature to increase at Manglillo	A	DNER, AZA	Miguel Canals	In kind	2003
Build interpretation hut at Manglillo	B	DNER, AZA	Miguel Canals	\$1.5K	2004
Identify and secure additional release sites in north and south and construct ponds where required	A	DNER, USFWS, AZA	Silmarie Padron	In kind	2004

Research Working Group Report

Synopsis: The greatest research needs for the recovery program are to identify which populations are **unique** and therefore most in need of conservation action *in* and *ex situ*, and to gather basic biologic data on representative *in situ* populations to provide necessary information for habitat and population management.

Objectives in order of sequence/priority

- 1) Identify unique populations; formalize/conduct population surveys in North and South
 - Exp and GIS project to characterize historic/current toad habitat for northern and southern populations
 - Contact Jamie to see if he would be interested if funding were obtained: Who? Bob Johnson
 - Identify other candidates if Jaime not interested: Who? Research Coordinator
 - Secure funding for this work (stipend, software, etc.): Who? Bob Johnson
 - Ensure security of people working in North around private land: Who? Quebradillas, Ernesto Estremera; elsewhere? Citizens of Karst?
 - Use this information to identify additional potential sites in North and South
 - Use GIS characterization to predict other localities: Who? Student/Researcher
 - Ground-test GIS predictions, i.e., visit sites to ensure they are realistic: Who? Student/Researcher
 - Monitor these historic/current/potential sites (develop protocol, organize/deploy monitoring team)
 - Write protocol: Who? Enrique Hernandez, Ernesto Estremera, Miguel Canals, Luis Nieves; invite feedback from DNER and USFWS
 - Construct illustrated guide to calls, nests, eggs, larvae, metamorphs of all anurans in area: Who? Toronto Zoo staff
 - Coordinate all parties (DNER, university, high school, citizens) into survey groups: Who? Research Coordinator
 - Raise local awareness in North to permit work near/on private lands: Who? Quebradillas, Ernesto Estremera, elsewhere? Citizens of Karst?
 - Ascertain precise weather parameters key to inducing immersion or breeding event to set standard for initiating survey protocol: Who? Miguel Canals
 - Monitor weather patterns at potential sites to know when to survey (might involve identifying key weather stations or obtaining/setting up monitoring equipment): Who? Research Coordinator
- 2) Identify unique populations; conduct phylogenetic analysis of all populations (extirpated and extant)
 - Push forward on genetic analyses: mtDNA, nuclear/microsatellite, and karyotype. What is needed by current researcher? Who? Kaela to do or identify someone to do

- Identify researcher to do morphometrics analysis: Who? Contact Greg Pregill? Compare N and S forms from pickled specimens by students of Fernando Bird, UPR Mayaguez, Rafael Joglar or Richard Thomas, UPR Rio Piedras
- Identify researcher to do call analysis: Who? Toronto Zoo staff has both types alive and could do or facilitate, add analysis from other zoos to evaluate intra- and interpopulation differences
- Identify researcher to do intergrade viability study: Who? Toronto Zoo staff has both types alive and could do or facilitate
- Identify researcher to do demography comparison

3) Empower PR researcher to study life history/demography/ecology of representative populations

- Identify student/researcher to cover each unique population identified in Objective 1: Who? Research Coordinator
 - Raise money for stipends and supplies: Who? Research Coordinator. AMP for undergrads, AAAS for grad students?
 - Identify what parameters need to be measured and how (sex ratios, survival across years, other Vortex-type parameters, habitat utilization, predator/prey relations, parasite/disease issues, interspecific competition with introduced anurans at all life stages, intraspecific tadpole density-dependent inhibition): Who? Research Coordinator

Priorities for Research

Habitat Information Needed

A. Establish the range of populations:

1. Northern:

a) Historical:

1) Natural collection sites:

- (a) Isabela (Quebrada La Sequia),
- (b) Quebradillas (Quebrada Bellaca?),
- (c) Bayamón (?)

2) Man-made collection sites: Quebradillas (Hoyo Brujo, Los Pagán)

3) Artificial release sites: Quebradillas, Cambalache

b) Current: Isabela (?), Quebradillas (?)

c) Potential: Isabela, Quebradillas, Finca Vale-Arecibo, Bosque Cambalache, Hacienda La Esperanza-Manatí, Bosque de Vega-Vega Alta

2. Southern:

a) Historical:

1) Natural collection sites: Coamo, Guánica,

2) Artificial collection sites:

3) Artificial release sites

b) Current: Guánica (Tamarindo)

c) Potential:

B. Characterize the habitat:

1. Physical and Chemical characterization

- a) Geologic substrate and soil composition of breeding pools;
 - b) Size and depth (maximum possible) of breeding pools;
 - c) Chemical characteristics of water in breeding pools;
 - d) Temperature and isolation potential of breeding pools;
2. Biotic characterization (per pool, at least in rainy and non-rainy seasons)
- a) Phytoplankton in breeding pools;
 - b) Zooplankton in breeding pools;
 - c) Algae and water plants in breeding pools;
 - d) Macroinvertebrates in breeding pools;
 - e) Vegetation growing adjacent to breeding pools;
 - f) Macroinvertebrates detected adjacent to breeding pools;
 - g) Vertebrates detected adjacent to breeding pools.
3. Geographical: Northern and Southern:
- a) Historical: Map all collection sites and compare (GIS);
 - b) Current: Map all sites and compare with historical;
 - 1) Potential: Identify through GIS potential collection or releasing sites (GIS)
- C. Develop a habitat evaluation protocol:
1. In private land:
- a) Determine precise location of site (GPS), measure it including buffer zone;
 - b) Determine position of site to other known breeding/collection sites;
 - c) Determine distance of site to closest protected land or zone;
 - d) Identify the owner(s) of land;
 - e) Explain to owner the importance of securing CT survival;
 - f) Explore owner's disposition to allow monitoring of CT in site;
 - g) Inquire the disposition of owner to protect land piece;
 - h) Obtain written authorization of owner to access site for study purposes;
 - i) Inform the landowner at least 24 hours prior to characterization visit;
 - j) Carry out characterization protocol;
 - k) Identify major risks to integrity of site;
 - l) Inform the landowner of findings.
2. In public land.
- a) Determine precise location of site (GPS), measure it including buffer zone;
 - b) Determine distance of site to closest known breeding/collecting sites;
 - c) Identify major risks to integrity of site;
 - d) Coordinate with personnel in charge scheduled visits to site;
 - e) Carry out characterization protocol;
 - f) Provide copy of data to government personnel overlooking the site.
- D. Develop a population census protocol:
1. In private land:
- a) Research rain patterns in the municipality/town/area where site is located;
 - b) Contact the landowner to inform intention of visiting area in rain season;
 - c) As critical meteorological parameters occur, schedule visit to the area;
 - d) Inform the landowner at least 24 hours prior to visits;
 - e) Have illustrated guide to nests, tadpoles and juveniles of anuran species;
 - f) Search for presence of adult anurans of any species (by sight or sound);

- g) Play CT songs for several minutes to identify response of males or females;
- h) Photograph any adult CT encountered, document its location and behavior;
- i) Identify and photograph any/each nest present;
- j) Estimate number of eggs/nest of each species;
- k) Take samples of tadpoles and identify the species with key;
- l) Estimate proportions of tadpoles of each species present at each visit;
- m) Estimate ages of tadpoles of each species in area;
- n) Identify presence of juvenile/froglets of each species in area;
- o) Inform the landowner of findings, don't let him/her in a blackout!

2. In public land:

- a) Research rain patterns in the municipality/town/area where site is located;
- b) As critical meteorological parameters occur, visit the area;
- c) Have illustrated guide to nests, tadpoles and juveniles of anuran species;
- d) Search for presence of adult anurans of any species (by sight or sound);
- e) Play CT songs for several minutes to identify response of males or females;
- f) Photograph any adult CT encountered, document its location and behavior;
- g) Identify and photograph any/each nest present;
- h) Estimate number of eggs/nest of each species;
- i) Take samples of tadpoles and identify the species with key;
- j) Estimate proportions of tadpoles of each species present at each visit;
- k) Estimate ages of tadpoles of each species in area;
- l) Identify presence of juvenile/froglets of each species in area;
- m) Inform government personnel in charge of findings; request random visits.

E. Develop a population monitoring protocol:

1. In private land.

- a) Inform the landowner at least 24 hours prior to visits;
- b) Visit the site every 3 to 7 days on a regular basis;
- c) Follow & graph metamorphosis schedule in each species, specially CT;
- d) Examine and map movements of froglets and juveniles leaving ponds;
- e) Spend a day to produce a behavioral ethogram for each species;
- f) Inspect surroundings for CT adults or juveniles and map any found;
- g) Document feeding or any other interspecific interaction involving CT;
- h) Carry out population census protocol until no anurans are detected;
- i) Inform the landowner of findings, don't let him/her in a blackout!

2. In public land.

- a) Visit the site every 3 to 7 days on a regular basis;
- b) Follow & graph metamorphosis schedule in each species, specially CT;
- c) Examine and map movements of froglets and juveniles leaving ponds;
- d) Spend a day to produce a behavioral ethogram for each species;
- e) Inspect surroundings for CT adults or juveniles and map any found;
- f) Document feeding or any other interspecific interaction involving CT;
- g) Carry out population census protocol until no anurans are detected;
- h) Inform government personnel in charge of / findings; request random visits.

Genetic/Taxonomic Information Needed:

- A. Document similarities/differences between karyotypes in N and S populations:
 - 1. Compare number of chromosomes in male/females of N&S;
 - 2. Contrast sizes of chromosomes from males & females of each N & S;
 - 3. Compare forms of chromosomes from males & females of each N & S; B.
- B. Compare genetic/biochemical differences between N & S populations:
 - 1. Compare mitochondrial DNA from specimens;
- C. Studies comparing the available variability/heterozygosity in captive populations.
- D. Detailed morphological studies in Crested Toads, emphasis in N and S populations:
- E. Detailed acoustic analyses of male vocalizations from captives: intrapopulations and interpopulations:
- F. Integration studies (potential for N & S hybrids).

Life History/Demography/Ecology Information Needed:

- A. Autecological studies of Crested Toads:
 - 1. Sex ratio of adult males to females in the wild.
 - 2. Time between deposition of eggs and enclosure of eggs in the wild.
 - 3. Comparison of pond size with number of nests, eggs deposited and froglet production in the wild.
 - 4. Comparison of timing of hatching between localities and between seasons.
 - 5. Behavior of tadpoles in the wild.
 - 6. Behavior of froglet/juveniles in the wild.
 - 7. Environmental cues inducing breeding behavior in captivity.
 - 8. Differential fitness between actual breeding sites.
 - 9. Determine hiding places in the wild.
 - 10. Compare hiding places of males and females.
 - 11. Compare hiding places of juveniles and adults.
- B. Synecological studies:
 - 1. Health assessment in the wild.
 - 2. Incidence of parasites in the wild.
 - 3. Diet of tadpoles in the wild.
 - 4. Diet of froglets in the wild (catalog, determine versatility of diet).
 - 5. Diet of adults in the wild (catalog, determine versatility, plasticity).

Captive Breeding Information Needed:

- 1. Sex ratio of males to females (in an age pyramid) in captivity
- 2. Proportion of eggs that hatch in captivity.
- 3. Proportion of males to females in eggs per nest.
- 4. Proportions of males to females to survive through tadpole stages.
- 5. Proportion of male to female in surviving froglet juveniles per nest.
- 6. Survivorship curves for male/female frogs in captivity.
- 7. Longevity in captivity of each sex (potential).
- 8. Time to reach maturity in each sex at captive North and South populations.
- 9. Reproductive potential (fertility changes) through age in both sexes.
- 10. Survivorship of each sex across the years in captivity (potential).

11. Compare pond size for complete development of nest of Crested Toads (Density dependence factors present? Can pond size ensures greater survivorship?).
12. Compare pond size or amount of water to days to complete metamorphosis.
13. Health assessment in captivity.
14. Incidence of parasites in captivity.
15. Comparative diet studies of *Bufo marinus*, *Leptodactylus albilabris*, *Osteopilus septentrionalis* and *Rana catesbiana* (Do -or can- any of these species, compete with Crested Toads for resources? For which resources? At which growth stages?)
16. Competition study of Crested Toads bred in tanks with B.m., L.a., O.S. and R.c. to check possible interspecific inhibition between any species to Crested Toads

Modeling Working Group Report

Population Viability Analysis of the Puerto Rican Crested Toad, *Peltophryne lemur*, using VORTEX

Background: Modeling and Population Viability Analysis

A model is any simplified representation of a real system. We use models in all aspects of our lives, in order to (1) extract the important trends from complex processes, (2) permit comparison among systems, (3) facilitate analysis of causes of processes acting on the system, and (4) make predictions about the future. A complete description of a natural system, if it were possible, would often decrease our understanding relative to that provided by a good model, because there is "noise" in the system that is extraneous to the processes we wish to understand. For example, the typical representation of the growth of a wildlife population by an annual percent growth rate is a simplified mathematical model of the much more complex changes in population size. Representing population growth as an annual percent change assumes constant exponential growth, ignoring the irregular fluctuations as individuals are born or immigrate, and die or emigrate. For many purposes, such a simplified model of population growth is very useful, because it captures the essential information we might need regarding the average change in population size, and it allows us to make predictions about the future size of the population. A detailed description of the exact changes in numbers of individuals, while a true description of the population, would often be of much less value because the essential pattern would be obscured, and it would be difficult or impossible to make predictions about the future population size.

In considerations of the vulnerability of a population to extinction, as is so often required for conservation planning and management, the simple model of population growth as a constant annual rate of change is inadequate for our needs. The fluctuations in population size that are omitted from the standard ecological models of population change can cause population extinction, and therefore are often the primary focus of concern. In order to understand and predict the vulnerability of a wildlife population to extinction, we need to use a model which incorporates the processes which cause fluctuations in the population, as well as those which control the long-term trends in population size (Shaffer 1981). Many processes can cause fluctuations in population size: variation in the environment (such as weather, food supplies, and predation), genetic changes in the population (such as genetic drift, inbreeding, and response to natural selection), catastrophic effects (such as disease epidemics, floods, and droughts), decimation of the population or its habitats by humans, the chance results of the probabilistic events in the lives of individuals (sex determination, location of mates, breeding success, survival), and interactions among these factors (Gilpin and Soulé 1986).

Models of population dynamics, which incorporate causes of fluctuations in population size in order to predict probabilities of extinction, and to help identify the processes, which contribute to a population's vulnerability, are used in "Population Viability Analysis" (PVA) (Lacy 1993/4). For the purpose of predicting vulnerability to extinction, any and all population processes that

impact population dynamics can be important. Much analysis of conservation issues is conducted by largely intuitive assessments by biologists with experience with the system. Assessments by experts can be quite valuable, and are often contrasted with "models" used to evaluate population vulnerability to extinction. Such a contrast is not valid, however, as *any* synthesis of facts and understanding of processes constitutes a model, even if it is a mental model within the mind of the expert and perhaps only vaguely specified to others (or even to the expert himself or herself).

A number of properties of the problem of assessing vulnerability of a population to extinction make it difficult to rely on mental or intuitive models. Numerous processes impact population dynamics, and many of the factors interact in complex ways. For example, increased fragmentation of habitat can make it more difficult to locate mates, can lead to greater mortality as individuals disperse greater distances across unsuitable habitat, and can lead to increased inbreeding which in turn can further reduce ability to attract mates and to survive. In addition, many of the processes impacting population dynamics are intrinsically probabilistic, with a random component. Sex determination, disease, predation, mate acquisition -- indeed, almost all events in the life of an individual -- are stochastic events, occurring with certain probabilities rather than with absolute certainty at any given time. The consequences of factors influencing population dynamics are often delayed for years or even generations. With a long-lived species, a population might persist for 20 to 40 years beyond the emergence of factors that ultimately cause extinction. Humans can synthesize mentally only a few factors at a time, most people have difficulty assessing probabilities intuitively, and it is difficult to consider delayed effects. Moreover, the data needed for models of population dynamics are often very uncertain. Optimal decision-making when data are uncertain is difficult, as it involves correct assessment of probabilities that the true values fall within certain ranges, adding yet another probabilistic or chance component to the evaluation of the situation.

The difficulty of incorporating multiple, interacting, probabilistic processes into a model that can utilize uncertain data has prevented (to date) development of analytical models (mathematical equations developed from theory) which encompass more than a small subset of the processes known to affect wildlife population dynamics. It is possible that the mental models of some biologists are sufficiently complex to predict accurately population vulnerabilities to extinction under a range of conditions, but it is not possible to assess objectively the precision of such intuitive assessments, and it is difficult to transfer that knowledge to others who need also to evaluate the situation. Computer simulation models have increasingly been used to assist in PVA. Although rarely as elegant as models framed in analytical equations, computer simulation models can be well suited for the complex task of evaluating risks of extinction. Simulation models can include as many factors that influence population dynamics as the modeler and the user of the model want to assess. Interactions between processes can be modeled, if the nature of those interactions can be specified. Probabilistic events can be easily simulated by computer programs, providing output that gives both the mean expected result and the range or distribution of possible outcomes. In theory, simulation programs can be used to build models of population dynamics that include all the knowledge of the system which is available to experts. In practice, the models will be simpler, because some factors are judged unlikely to be important, and because the persons who developed the model did not have access to the full array of expert knowledge.

Although computer simulation models can be complex and confusing, they are precisely defined and all the assumptions and algorithms can be examined. Therefore, the models are objective, testable, and open to challenge and improvement. PVA models allow use of all available data on the biology of the taxon, facilitate testing of the effects of unknown or uncertain data, and expedite the comparison of the likely results of various possible management options.

PVA models also have weaknesses and limitations. A model of the population dynamics does not define the goals for conservation planning. Goals, in terms of population growth, probability of persistence, number of extant populations, genetic diversity, or other measures of population performance must be defined by the management authorities before the results of population modeling can be used. Because the models incorporate many factors, the number of possibilities to test can seem endless, and it can be difficult to determine which of the factors that were analyzed are most important to the population dynamics. PVA models are necessarily incomplete. We can model only those factors which we understand and for which we can specify the parameters. Therefore, it is important to realize that the models probably underestimate the threats facing the population. Finally, the models are used to predict the long-term effects of the processes presently acting on the population. Many aspects of the situation could change radically within the time span that is modeled. Therefore, it is important to reassess the data and model results periodically, with changes made to the conservation programs as needed.

VORTEX Population Viability Analysis Model

For the analyses presented here, the VORTEX (Lacy, et. al 2003) computer software (Lacy 1993a) for population viability analysis was used. VORTEX models demographic stochasticity (the randomness of reproduction and deaths among individuals in a population), environmental variation in the annual birth and death rates, the impacts of sporadic catastrophes, and the effects of inbreeding in small populations. VORTEX also allows analysis of the effects of losses or gains in habitat, harvest or supplementation of populations, and movement of individuals among local populations.

Density dependence in mortality is modeled by specifying a carrying capacity of the habitat. When the population size exceeds the carrying capacity, additional mortality is imposed across all age classes to bring the population back down to the carrying capacity. The carrying capacity can be specified to change linearly over time, to model losses or gains in the amount or quality of habitat. Density dependence in reproduction is modeled by specifying the proportion of adult females breeding each year as a function of the population size.

VORTEX models loss of genetic variation in populations, by simulating the transmission of alleles from parents to offspring at a hypothetical genetic locus. Each animal at the start of the simulation is assigned two unique alleles at the locus. During the simulation, VORTEX monitors how many of the original alleles remain within the population, and the average heterozygosity and gene diversity (or “expect heterozygosity”) relative to the starting levels. VORTEX also monitors the inbreeding coefficients of each animal, and can reduce the juvenile survival of inbred animals to model the effects of inbreeding depression.

VORTEX is an *individual-based* model. That is, VORTEX creates a representation of each animal in its memory and follows the fate of the animal through each year of its lifetime.

VORTEX keeps track of the sex, age, and parentage of each animal. Demographic events (birth, sex determination, mating, dispersal, and death) are modeled by determining for each animal in each year of the simulation whether any of the events occur. Events occur according to the specified age and sex-specific probabilities (i.e., binomial processes are simulated). Demographic stochasticity is therefore a consequence of the uncertainty regarding whether each demographic event occurs for any given animal.

In *population-based* models (which VORTEX is not), the model tracks the total numbers of animals in each age and sex class, but not the fates of individuals. The numbers in the subsequent time steps are determined by using theoretical equations, which predict the changes in population size from the mean demographic rates. Demographic stochasticity is often modeled by letting the demographic rates used in the equations fluctuate, or by adding a correction factor which modifies the final population sizes by amounts sampled from theoretical distributions that describe the expected amount of demographic stochasticity.

Individual-based models, like VORTEX, can be thought of as being “bottom-up” models, in which population dynamics are modeled by simulating the demographic events (e.g., births, dispersal, deaths) which generate those dynamics. In contrast, a more “top-down” approach would use equations derived from theory to predict the population changes which would result from the specified demographic rates. “Bottom-up” models can usually be made to model very complex and interacting processes, but can be rather slow to run. “Top-down” models are usually faster and more general, but rely on the accuracy of theoretical equations that are based on simplifying assumptions, which may not always be appropriate.

VORTEX requires lots of population-specific data, rather than using ecological theory to generate many parameters describing population processes. For example, the user must specify the amount of annual variation in each demographic rate caused by fluctuations in the environment. In addition, the frequency of each type of catastrophe (drought, flood, epidemic disease) and the effects of the catastrophes on survival and reproduction must be specified. Rates of migration (dispersal) between each pair of local populations are specified, rather than being assumed to be a simple function of distance or other parameters. Because VORTEX requires specification of many biological parameters, it is not necessarily a good model for the examination of population dynamics that would result from some generalized life history. It is perhaps more usefully applied to the analysis of a specific population in a specified environment.

The VORTEX outputs are robust, including text, tables and graphs. The program is written in the C programming language with a Microsoft Visual Basic user-interface for data input, analysis, and output development. VORTEX is not copy protected, and users are encouraged to provide copies of the disk and manual to any other people who might have a need for the program. It is available on the Internet at <http://www.vortex9.org/vortex.html>. Further information on VORTEX is available in Lacy (1993a) and Lacy et al. (1995).

Dealing with uncertainty

Estimates of the key biological parameters used by the VORTEX model were provided by participants at the workshop on the Puerto Rican crested toad, which was held in Puerto Rico in October 2003. Some of the values were readily obtained from field data, and there was little

uncertainty in the appropriate value to use for modeling. For some other parameters, different biologists at the workshop had different information, or interpreted the same data in different ways, and consequently had divergent views on what values best described the population of PRCT. For some other parameters, there are no quantitative data, and the field biologists had to make guesses (often, after some arm-twisting) based on general knowledge of the species and habitat. When there was uncertainty regarding an important biological parameter, analyses were run with alternative plausible values (“sensitivity testing”).

It is important to recognize that uncertainty regarding the biological parameters occurs at several levels and for independent reasons. Uncertainty can occur because the parameters have never been measured on the population. Uncertainty can occur because limited field data have yielded estimates with potentially large sampling error. Uncertainty can occur because independent studies have generated discordant estimates. Uncertainty can occur because environmental conditions or population status have been changing over time, and field surveys were conducted during periods that may not be representative of long-term averages. Uncertainty can occur because the environment will change in the future, so that measurements made in the past may not accurately predict future conditions.

Sensitivity testing is necessary to determine the extent to which uncertainty in input parameters results in uncertainty regarding the future fate of the PRCT populations. If alternative plausible parameter values result in divergent predictions for the population, then it is important to try to resolve the uncertainty with better data. Sensitivity of population dynamics to certain parameters also indicates that those parameters describe factors, which could be critical determinants of population viability. Such factors are therefore good candidates for efficient management actions designed to ensure the persistence of the population.

The above kinds of uncertainty should be distinguished from several more sources of uncertainty about the future of the population. Even if long-term average demographic rates are known with precision, variation over time caused by fluctuating environmental conditions will cause uncertainty in the fate of the population at any given time in the future. Such environmental variation should be incorporated into the model used to assess population dynamics, and will generate a range of possible outcomes (perhaps represented as a mean and standard deviation) from the model. In addition, most biological processes are inherently stochastic, having a random component. The stochastic or probabilistic nature of survival, sex determination, transmission of genes, acquisition of mates, reproduction, and other processes preclude exact determination of the future state of a population. Such demographic stochasticity should also be incorporated into a population model, because such variability both increases our uncertainty about the future and can also change the expected or mean outcome relative to that which would result if there were no such variation. Finally, there is “uncertainty” which represents the alternative actions or interventions, which might be pursued as a management strategy. The likely effectiveness of such management options can be explored by testing alternative scenarios in the model of population dynamics, in much the same way that sensitivity testing is used to explore the effects of uncertain biological parameters.

Questions to be explored

Below are some of the interrelated questions and issues which discussed at the workshop and

which have begun to be explored by PVA simulation modeling.

Using the best current information on the biology of the taxon and its habitat, is the only known natural occurring population at Tamarindo projected to persist if all conditions remain as they are now? If the population is at risk of extinction, is the extinction expected to result primarily from negative average population growth (mean deaths exceeding mean births), from large fluctuations in numbers, from an inability to recover from periodic catastrophic declines, from effects of accumulated inbreeding, or from a combination of these factors? Beyond just the persistence of the population, is the expected rate of loss of genetic variation within the limits that are deemed acceptable? Which factors have the greatest influence on the projected population performance? If important factors are identified, management actions might be designed to improve these factors or ameliorate the negative effects.

How would the metapopulation respond (in numbers and in probability of persistence) to the following possible management actions?

- Increase the number of subpopulations in the metapopulation
- Maintain supplementation of release populations for 10 or 20 years
- Establish corridors that allow animal to migrate between subpopulations

Input Parameters Puerto Rican Crested Toad: Overview of Current Status

PRCT were once distributed more widely throughout Puerto Rico. In the 1980's, two distinct extant populations were identified: One in the northern part of the island at Quebradillas, and one in the south at the Guánica Commonwealth Forrest. Genetic work has suggested that these two populations are distinct, although this work is not completely conclusive. The Northern population appears to have become extinct by the early 1990's and the only representatives of that population are a few remaining toads in captivity, all from a single lineage of siblings. The wild Southern population still persists and breeding is frequently observed. Southern toads are well represented in the captive population. With no known extant wild populations of Northern toads, the VORTEX analysis at this PHVA workshop examined only the Southern toads and their management.

The Southern Puerto Rican Crested toads exist in three distinct subpopulations that form an intensely managed metapopulation. First, there is the last known naturally existing population of PRCT at Tamarindo, PR. DRNA Guánica Commonwealth Forest personnel manage this population to facilitate toad survival and reproduction. Second, there is a captive population managed by the American Zoo and Aquarium Association (AZA) Species Survival Plan (SSP). This population is used to assure survival of PRCTs in captivity in the event of catastrophic loss of the Tamarindo population and as a resource to produce PRCTs for release in new areas. Finally, there are two additional populations that have been created from released animals produced in the SSP captive population. During the last year, one of these release sites has had reproduction of free-ranging toads.

Special consideration for a fecund species

Using individual-based models such as VORTEX to examine a fecund species such as a toad

presents challenges. Clutches of toads may range in the thousands, with a correspondingly high mortality for the first two months. It was considered that at least 50% of the hatched larvae die or fail to metamorphose. Once the toadlets leave the breeding site, they must avoid predation and find appropriate refugia to survive the harsh dry environment. Participants considered that on the average, 150 animals from each clutch would survive to 30 days post metamorphosis. For the VORTEX model, this was considered the start of life with a mean of 150 toadlets produced per breeding pair. Variance in clutch size was considered high and a SD of 75 was used to model the numbers produced in each clutch. Thus, the number of surviving toadlets per pair would typically be from 0 to 300 (i.e., ± 2 SD, the range expected for about 95% of the pairs).

General characteristics of the PRCT

Impact of any inbreeding: It is not known what impact inbreeding would have on survival and fecundity of PRCT. All current Southern PRCTs were restricted to one breeding site (Tamarindo) where inbreeding (mating between close relatives) may have occurred. The consensus of the group at the PHVA is that inbreeding is probably less important a factor in amphibians and two recessive lethal alleles was considered a reasonable estimate of the average for PRCT. There are few data on the average number of lethal alleles per individual for any amphibian to support or refute this assumption. This value was used in all simulations. All inbreeding depression was considered the result of recessive lethals, in which the lethal alleles assigned to founder animals are each unique, and these recessive lethal alleles could be removed by natural selection (through the death of inbred, homozygous animals). Thus, if inbreeding does occur, the effects of inbreeding would diminish through the generations, as the population became purged of its lethal alleles.

General characteristics of the Tamarindo population

Age of first reproduction (parturition) of females: VORTEX assumes that females all become sexually mature at the same age, and that fecundity is constant thereafter (until the animal dies). Female PRCT can probably reproduce at three years of age in the wild. Fifteen years of age was considered the age of reproductive senescence, although with the high mortality rates of this species, few toads will make it to this age.

Age of first reproduction of males: Males were assumed to begin breeding at 2 years of age.

Density dependence: Except for a "ceiling" maximum (Carrying Capacity K) set on the number of animals that could be maintained within each population, no attempt was made to model any possible effects of increased population density on breeding or mortality rates. If there is density dependence, then the population might grow more slowly as it approaches the carrying capacity of the habitat.

Mortality and mortality environmental variation rates: See tables below

Mortality Rates for Females

Age	Mortality	Mortality EV (as SD)
0 to 1	50	10
1 to 2	50	10
2 to 3	55	10
3 and above	55	10

Mortality Rates for Males

Age	Mortality	Mortality EV (as SD)
0 to 1	50	10
1 to 2	50	10
2 and above	50	10

Catastrophes: VORTEX models catastrophe years as a distinct phenomenon from the more typical environmental variation in demographic rates. Hence, the variation resulting from catastrophes (see below) was imposed on top of the environmental variation that was modeled. Experts at the PHVA considered that there were five separate types of catastrophes that effect the Tamarindo population (see table). Although there are few data on which to estimate the frequencies and severities of catastrophes, the kinds of catastrophes discussed include severe storms that cause salt-water flooding of breeding sites and can wash breeding toads out to sea; droughts that can kill adults and young toadlets; chemical (e.g., automobile oil) contamination of breeding sites that kill tadpoles; and disease.

Hypothesized Catastrophes Affecting Wild Populations

Frequency	Effect on Reproduction	Effect on Mortality	Global or Local
10%	1	0.75	Local
1%	0	0.5	Local
1%	0	0.25	Local
5%	1	0.75	Local
2%	0.5	1	Local

Maximum age: VORTEX assumes that animals can keep breeding (with normal fecundity) up to the time they die. PRCTs are thought to decline in condition and have reduced fecundity after about 15 years of age. For the analyses, the maximum age was set at 15 years.

Breeding system: PRCT are polygamous. For the VORTEX simulation modeling, it was assumed that in each year 95% of the adult males (those over 2 years of age) are in the pool of breeding males. VORTEX randomly selects which adult males are in that breeding pool each year of the simulation, and it assigns females to the breeding males at random.

Fecundity: Participants considered that on the average 150 animals would survive for 30 days post metamorphosis (see “Special consideration for a fecund species” above). For the VORTEX model, this was considered the start of life with a mean of 150 toadlets produced per clutch. Variance in clutch size was considered high, and a SD of 75 was used to model the numbers produced in each clutch.

The numbers of females reproducing each year significantly affects the population dynamics. In the case of the PRCT, breeding occurs in discrete events, a maximum of once or twice a year. In some years, breeding does not occur at all. Workshop participants provided data for the Tamarindo population over the last 20 years (Canal, 2003). It appears that 25% of the years there is no reproduction, 50% of the years 60% of the females reproduce, and 25% of the years 90% of the females reproduced. The following Boolean function was used in VORTEX to estimate the number of females breeding each year:

$$[\text{Function}=(\text{SRAND}(Y+(R*1000))>.25)*60+((\text{SRAND}(Y+(R*1000))>.75)*30)]$$

Carrying capacity: Establishing carrying capacity for a secretive animal such as a toad is problematic and no real data exists. The participants felt that the Tamarindo area (including two smaller adjacent ponds) could sustain a carrying capacity (K) of 1200.

Initial Population Size: Based upon the estimates of the workshop participants, the initial population size for the Tamarindo population was set at 400 toads in a stable-age distribution.

General Characteristics of the Captive Population

Data for captive population was taken directly from AZA Studbook for PRCT (updated 2003).

Age of first reproduction (parturition) of females: VORTEX assumes that females all become sexually mature at the same age, and that fecundity is constant thereafter (until the animal dies). Female PRCT can reproduce at three years of age in captivity. Fifteen years of age was considered the age of reproductive senescence, although few toads will make it to this age.

Age of first reproduction of males: Males were assumed to begin breeding at two years of age.

Density dependence: Except for a "ceiling" maximum set (Carrying Capacity K) on the number of animals that could be maintained within each population, no attempt was made to model any possible effects of increased population density on breeding or mortality rates. If there is density dependence, then the population might grow more slowly as it approaches the carrying capacity of the habitat.

Mortality and mortality environmental variation rates:

Mortality Rates for Captive Females

Age	Mortality	Mortality EV (as SD)
0 to 1	.26	5
1 to 2	.16	5

2 to 3	.15	3
3 and above	.10	2

Mortality Rates for Captive Males

Age	Mortality	Mortality EV (as SD)
0 to 1	.26	5
1 to 2	.14	3
2 and above	.18	4

Catastrophes: VORTEX models catastrophe years as a distinct phenomenon from the more typical environmental variation in demographic rates. Hence, the variation resulting from catastrophes was imposed on top of the environmental variation that was modeled. For the captive population, only one catastrophe was modeled. This was a disease event that would occur with a 10% frequency and reduce reproduction by 25% and decrease survivability by 50%.

Maximum age: VORTEX assumes that animals can keep breeding (with normal fecundity) up to the time they die. PRCT are thought to decline in condition and have reduced fecundity after about 15 years of age. For the analyses, the maximum age was set at 15 years.

Breeding system: PRCT are polygamous. For the VORTEX simulation modeling, it was assumed that in each year 95% of the adult males (those over 2 years of age) are in the pool of breeding males. VORTEX randomly selects which adult males are in that breeding pool each year of the simulation, and it assigns females to the breeding males at random.

Fecundity: Most of the toads produced in captivity are designated for release at one or more sites. Animals are transported as larvae within a few weeks of hatching. For this reason, the same standard was applied for captive produced offspring as used for their wild produced counterparts (i.e., 150 animals would survive for 30 days post metamorphosis). For the VORTEX model, this was considered the start of life with a mean of 150 toadlets produced per clutch. Under controlled captive conditions, variance was estimated to be less than what was expected in wild breedings. A SD of 50 was used to model the numbers produced in each clutch.

Under controlled captive conditions, 10% of the females were considered in the breeding pool each year, with a SD of 3.

Initial population size and carrying capacity: The initial population size for the captive population was set at 403 distributed as indicated in the studbook. The carrying capacity (K) for captivity was set at 400.

Supplementation of captive population: Starting in 2007, the captive population was supplemented with 9 unrelated individuals every five years. These additional animals would be acquired as larvae from different clutches of eggs at Tamarindo. The harvest of these tadpoles was considered not to have any significant impact on the Tamarindo population. Supplementation did not occur unless there were at least 100 juveniles produced at Tamarindo in

the designated supplementation year.

General Characteristics of the Released Populations

The general characteristic of all released populations is based on parameters observed at Tamarindo with a few exceptions. The carrying capacity (K) for all released populations was established at 1000. In addition, all catastrophes were considered global (occurring for all wild populations), except number 5. These were considered to be localized events, which would occur at similar rates, but not necessarily linked to other catastrophes at other populations.

Modeling Particulars: Duration, Number of Iterations, Output Results

The different scenario populations were modeled for 100 years. Each scenario was simulated 1500 times in order to produce consistent estimates of the mean result and the variability in results. Results reported for each scenario include:

Stochastic r: The mean rate of stochastic population growth or decline demonstrated by the simulated populations, averaged across years and iterations, for all those simulated populations that are not extinct. This population growth rate is calculated each year of the simulation, prior to any truncation of the population size due to the population exceeding the carrying capacity. Usually, this stochastic *r* will be less than the deterministic *r* predicted from birth and death rates. The stochastic *r* from the simulations will be close to the deterministic *r* if the population growth is steady and robust. The stochastic *r* will be notably less than the deterministic *r* if the population is subjected to large fluctuations due to environmental variation, catastrophes, or the genetic and demographic instabilities inherent in small populations.

PE: The probability of population extinction, determined by the proportion of 500 populations of that scenario, which have gone extinct in the simulations. “Extinction” was defined in the VORTEX model as the lack of either sex.

Gene diversity (GD): The gene diversity or expected heterozygosity of the extant populations, expressed as a percent of the initial gene diversity of the population. Fitness of individuals usually declines proportionately with gene diversity (Lacy 1993b), with a 10% decline in gene diversity typically causing about 15% decline in survival of captive mammals (Ralls et al. 1988). Impacts of inbreeding on wild populations are less well known, but may be more severe than those observed in captive populations (Jiménez et al. 1994). Adaptive response to natural selection is also expected to be proportional to gene diversity. Long-term conservation programs often set a goal of retaining 90% of initial gene diversity (Soulé et al. 1986). Reduction to 75% of gene diversity would be equivalent to one generation of full-sibling or parent-offspring inbreeding.

Mean TE: The mean time for extinctions for iterations that did go extinct.

Parameters varied for Tamarindo for sensitivity testing

Sensitivity testing was performed on the Tamarindo population. This population was chosen for testing because it represented the only non-supplemented wild population and was the only wild population that has continued to persist since its discovery in the 1980's. The release

populations are supplemented several times a year with animals produced in captivity. It is assumed that, when established, the release populations will perform in a similar manner to the Tamarindo population (with the exception of having a reduced carrying capacity).

The sensitivity of the Tamarindo population to changes in four variables was explored. These include carrying capacity (K), an increase in mortality by 10%, inbreeding depression, and the elimination of catastrophe effects. The effects are presented in the table below.

Results of Sensitivity Testing

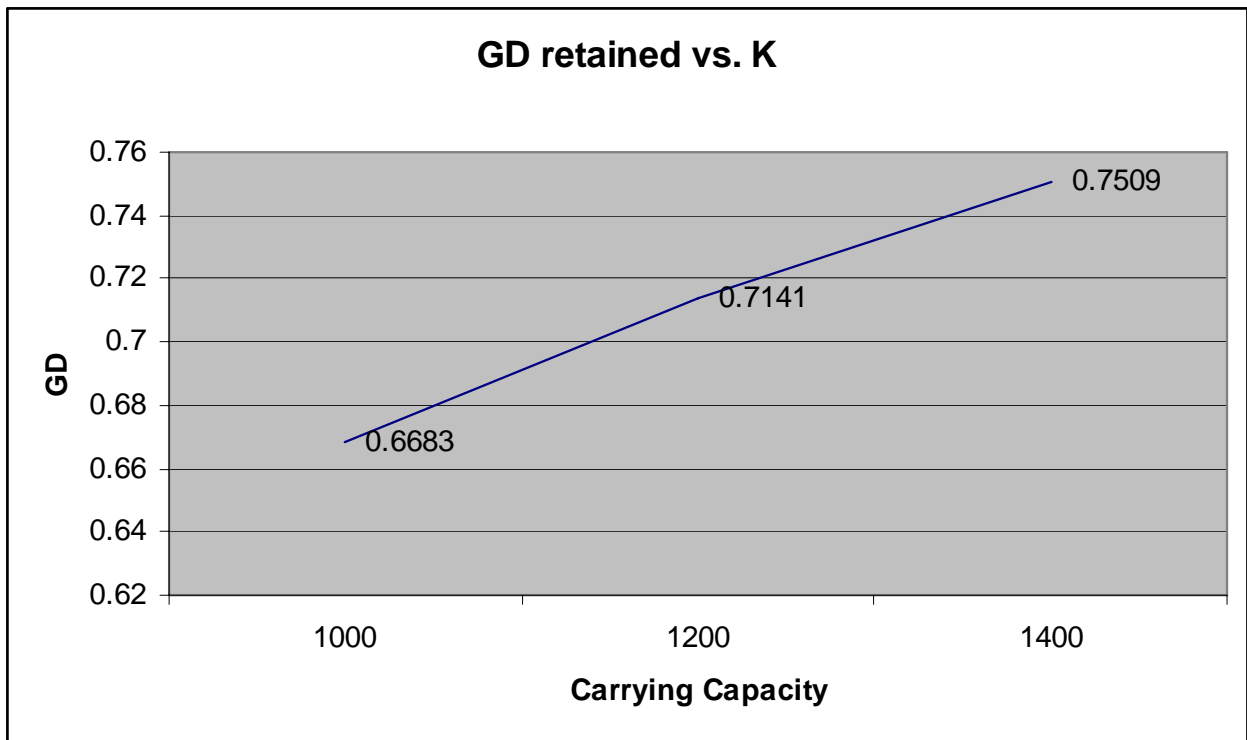
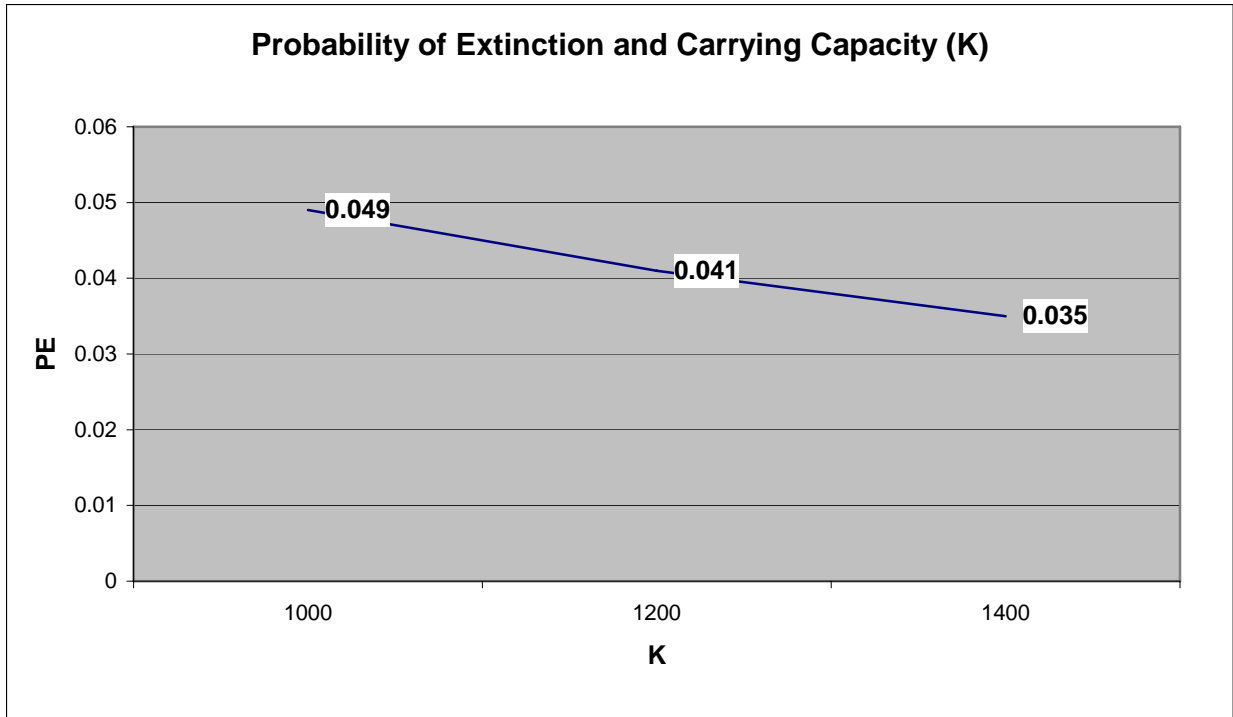
Table 1 Sensitivity Analysis Results

Scenario	Population	stoc-r	SD(r)	PE	GD	SD(GD)	MeanTE
Base line scenario K=1200	Tamarindo	0.441	1.349	0.041	0.7141	0.1187	53.9
Decrease K to 1000	Tamarindo	0.440	1.352	0.049	0.6683	0.1327	54.2
Increase K to 1400	Tamarindo	0.442	1.348	0.035	0.7509	0.1013	54.5
10% increase in all mortality rates	Tamarindo	0.324	1.393	0.136	0.6522	0.1451	47.5
Remove all effects of Catastrophes	Tamarindo	0.521	1.297	0.004	0.763	0.0834	53.8
No inbreeding effects	Tamarindo	0.452	1.362	0.035	0.7104	0.1194	48.7
Double inbreeding effects to 4 lethal alleles per individual	Tamarindo	0.433	1.337	0.044	0.7257	0.1080	46.0

Complete results of all Scenarios are presented in Appendix 1

Baseline scenario: The results from the parameters used to model the current Tamarindo population indicate a probably of extinction over 100 years at 0.041. This probability of extinction is high enough to be a concern. Currently, there is no immigration into this site. If it does go extinct, it will require a managed reintroduction effort. Efforts to mitigate negative stochastic events have been ongoing for years (Canal, Pers. Comm.). Mitigation has included closing of the breeding area to vehicular traffic, adding refugia for newly metamorphosed toadlets, the installation of a sea wall, and the addition of water to prevent temporary ponds from drying up prior to the emergence of toadlets.

Changes to carrying capacity: Values of the carrying capacity (K) of the Tamarindo area for PRCT are estimates. For the baseline Tamarindo model, 1200 total individuals were used. Sensitivity of the model to different values of K was tested with 1000 and 1400 individuals. Carrying Capacity (K) was inversely related to the probability of extinction PE (see chart below). Increased K lowers PE. There is also a decline in GD with a decrease in carrying capacity (K) (see chart below). If the true K for the Tamarindo population is substantially different from the 1200 used in the model, the model will not provide accurate PE values. These results identify an area where additional research could benefit modeling to develop future management plans for the PRCT.

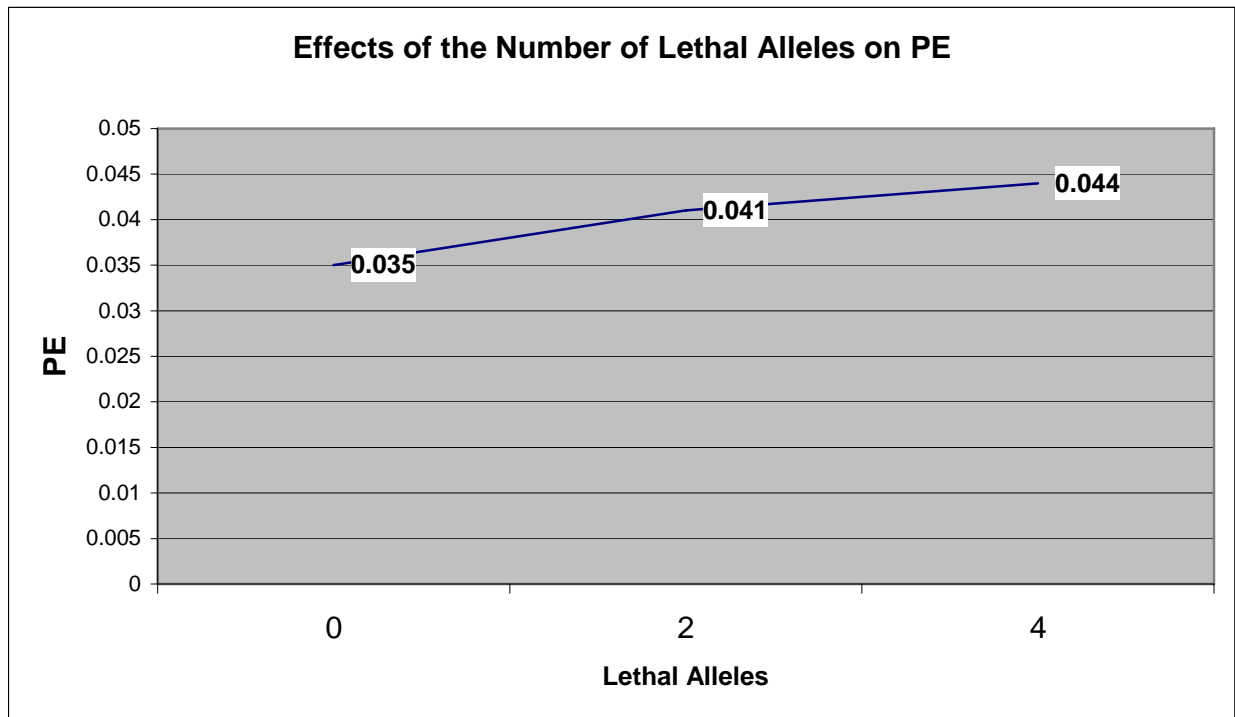


Increased mortality: Mortality rates used in this model were rough estimates. No empirical data is available on survival of wild PRCTs. A 10% increase in mortality rates of all age-classes results in a three-fold increase in the 100-year probability of extinction from 0.041 to 0.136. The model suggests that changes in mortality rates could have a significant impact on the Tamarindo

population. If mortality rates are only 10% higher than originally estimated, the population is at much higher risk of extinction. These results identify an area where additional research could benefit modeling to develop future management plans for the PRCT.

Elimination of Catastrophes: The effects of the five catastrophes outlined in the model were explored. When the effects of these events are removed from the model, the PE drops significantly from 0.041 to 0.004. This indicates that populations of PRCT have some difficulty recovering from negative stochastic events. As mentioned above under the base line scenario, efforts have focused on mitigating some of these events. These management techniques are probably of great value to the survival of this species at Tamarindo and other wild populations.

Effects of Inbreeding: Participants at the workshop felt that an average of 2 lethal alleles per individual was a reasonable estimate for the potential of inbreeding depression in PRCTs. This is less than the average for many mammal species of 3.14 per individual (Ralls et al. 1988). To determine the potential effects of number of lethal alleles on the PRCT, simulations were run with zero, two, and four lethal alleles per individual (see chart below). As expect, there is a direct relationship between the number of lethal alleles and the PE for the population. This effect appears to be less than one percent and is not as significant as effects from changes in K and mortality, as well as the catastrophes modeled. This figure assumes that all inbreeding depression is from recessive alleles that can be selected against and removed from the population.



Summary of sensitivity analysis for Tamarindo: From these sensitivity analyses, three of the tested factors appear to be more significant to the survival of the toad. First, a small general increase (10%) in mortality rates for the population can significantly raise the PE for PRCT. Second, the stochastic negative events (catastrophes) that are expected for the population do significantly increase the probability of extinction. Elimination of these catastrophes reduces the PE almost to zero. Finally, the carrying capacity (K) of the environment can impact the survival of the toad.

Captive population

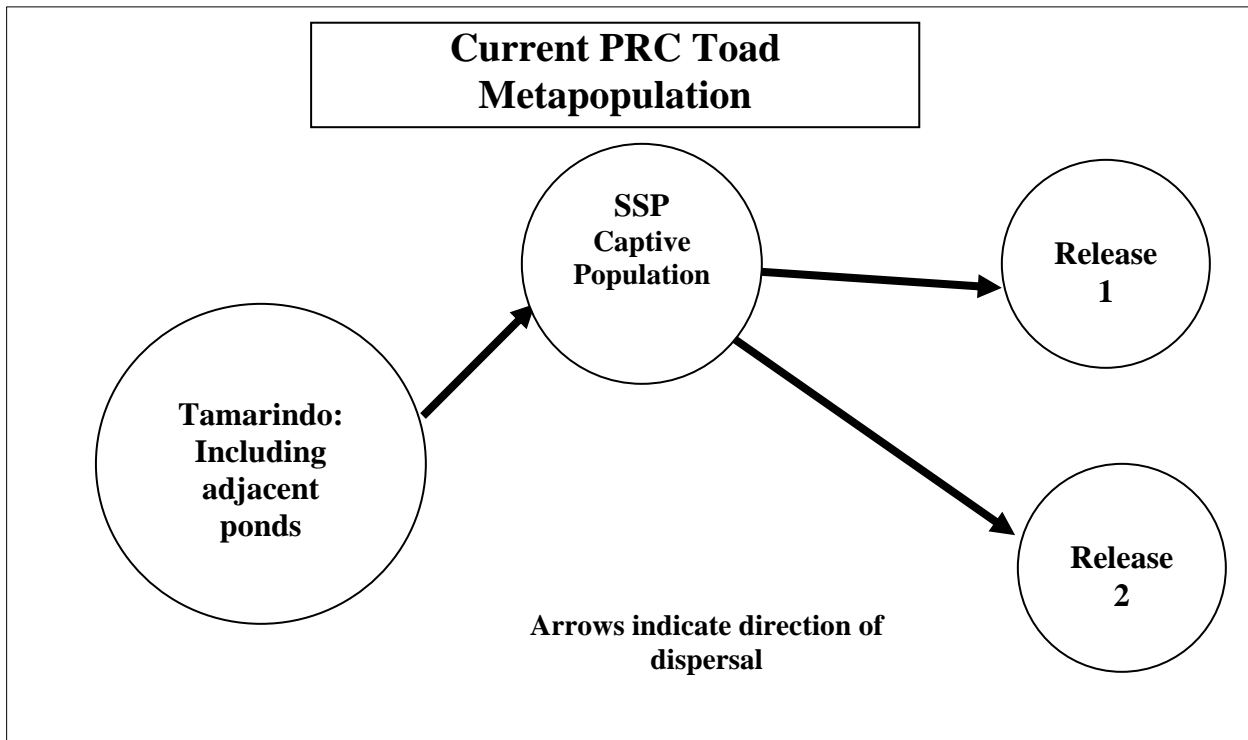
VORTEX analysis of the captive population indicates that there is little probability that a well-managed captive population will go extinct in the next 100 years. If managed with an influx of new diversity from the Tamarindo population, the average GD expected after 100 years is 0.8173 of the starting level.

Table 2 Captive Population

Population	stoc-r	SD®	PE	GeneDiv	SD(GD)
Captive	0.255	0.345	0	0.8173	0.0677

Development of Metapopulation Models

There is an extensive captive population program through the AZA (SSP®) for the PRCT. This captive population was first established in early 1980's. In the 1990's, offspring have been produced in captivity to establish additional wild populations near, but separate to the Tamarindo population. These populations are at a significant enough distance to prevent natural dispersal between sites. This was done to prevent the possible transfer of disease from released animals to the last remaining wild population at Tamarindo. Current two release sites, the captive population, and the Tamarindo population are all part of the metapopulation for crested toads. (see diagram below)



Note that there is dispersal in only one direction. This directional flow means subpopulations that go extinct will be re-colonized only if they are the recipients of individuals from another subpopulation (to the left). As an example, if Tamarindo subpopulation disappears, it would not be re-colonized under the management activities taking place currently.

General Characteristics of the Metapopulation

The release populations were based on data from the Tamarindo population with a lower carrying capacity (K) of 1000 and with supplementation from the captive produced toads.

Metapopulation Scenarios Explored

Five separate metapopulation scenarios were explored with VORTEX:

1. *Scenario 1* is based upon a single release site. There are three subpopulations: Tamarindo, Captivity, and a Release. Every five years, nine larvae will be collected at Tamarindo and added to the captive population. Animals are being bred in captivity and are being released. For this model, approximately 75 percent of the toads hatched each year in the captive SSP population will be released (see Table 5 below). These releases will continue for the full 100 years of the scenario.
2. *Scenario 2* is similar to the first, with the exception that the release program will be discontinued after 10 years. This scenario will test if the release populations remain viable after releases are discontinued.
3. *Scenario 3* is similar to the first, with the exception that the release program will be discontinued after 20 years. As in Scenario 2, this scenario will test if the release

populations remain viable after releases are discontinued.

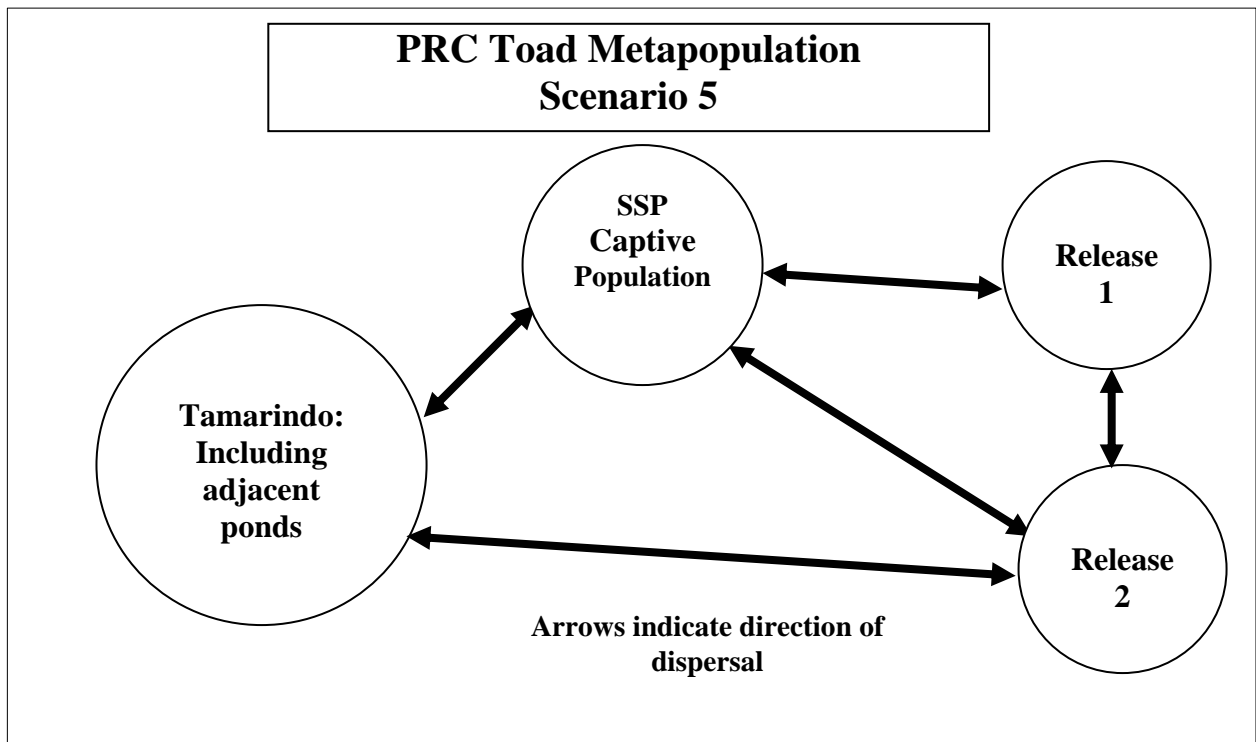
4. *Scenario 4* is based upon two release subpopulations. This scenario is close to the current management situation for the PRCT at the Guánica Commonwealth Forest. Similar to the first release site, this site has a carrying capacity (K) of 1000 for 30-day post-metamorphosis individuals. Instead of 75% of the annually captive production being released to only one site, animals are split evenly between the two sites (see below).
5. *Scenario 5* is based on the values in Scenario 4 with the addition of the supplementation of two adult pairs to each wild subpopulation annually. This supplementation represents the migration of these individuals between subpopulations, which allows augmentation and re-colonization if a population goes extinct.

Dispersal Rates for Scenarios 1, 2, and 3

	Tamarindo	Captive	Release
Tamarindo		0.00	0.00
Captive	0.00		75.00
Release	0.00	0.00	

Dispersal Rates for Scenario 4

	Tamarindo	Captive	Release 1	Release 2
Tamarindo		0.00	0.00	0.00
Captive	0.00		37.50	37.50
Release 1	0.00	0.00		0.00
Release 2	0.00	0.00	0.00	



Results of Metapopulation Scenarios

The results of these analyses are presented in the table below. In Scenarios 1-4, the captive SSP population is the only population that seems to be assured to persist. The SSP population is judiciously managed and is not exposed to the same catastrophic events that cause large variances in population numbers seen the wild. In Scenario 1, the release population is continually replenished with captive produced offspring for the full 100 years. This prevents this release population from becoming extinct, although the Tamarindo population may go extinct. In Scenarios 2 and 3, the introductions from captivity are ended at 10 and 20 years respectively. From these times on, the populations must perpetuate themselves. With similar parameters to the Tamarindo population, these populations have similar probabilities of extinction. The same is true with Scenario 4 with two release sites.

Only in Scenario 5 do all the populations persist. This is due to the migration of animals between the subpopulations. This model provides greater stability, but does allow a pandemic disease event to occur that could decimate all populations simultaneously.

Metapopulation Results

Scenario	Subpopulation	stoc-r	SD(r)	PE	GeneDiv	SD(GD)	MeanTE
Tamarindo - Baseline	Tamarindo	0.44	1.35	0.041	0.714	0.119	54
1) Metapopulation with 1 release site, continuous release	Tamarindo	0.44	1.33	0.036*	0.729	0.104	52
	Captive	0.26	0.35	0	0.817	0.068	
	Release 1	0.63	0.68	0	0.814	0.068	
	Metapopulation	0.58	0.75	0	0.880	0.045	
	Within Populations	0.44	0.79	0.012	0.787	0.080	52
2) Metapopulation with 1 release site, 10 yr. release	Tamarindo	0.44	1.33	0.032*	0.726	0.109	42
	Captive	0.54	0.52	0	0.595	0.159	
	Release 1	0.46	1.29	0.043	0.699	0.125	57
	Metapopulation	0.59	0.94	0	0.869	0.065	
	Within Populations	0.48	1.05	0.025	0.673	0.131	50
3) Metapopulation with 1 release site, 20 yr. release	Tamarindo	0.44	1.33	0.027*	0.724	0.116	53
	Captive	0.51	0.52	0	0.613	0.159	
	Release 1	0.48	1.23	0.039	0.708	0.122	61
	Metapopulation	0.59	0.92	0	0.870	0.064	
	Within Populations	0.48	1.03	0.022	0.682	0.132	57
4) Metapopulation with 2 release sites, 20 yr. release	Tamarindo	0.44	1.33	0.029*	0.725	0.109	44
	Captive	0.51	0.51	0	0.613	0.156	
	Release 1	0.47	1.25	0.027	0.711	0.119	61
	Release 2	0.47	1.25	0.033	0.713	0.113	64
	Metapopulation	0.58	0.98	0	0.899	0.048	
	Within Populations	0.47	1.09	0.022	0.690	0.124	56
5) Metapopulation with 2 release sites, 20 yr. release and migration between sites	Tamarindo	0.46	1.29	0			
	Captive	0.51	0.51	0			

	Release 1	0.49	1.21	0			
	Release 2	0.49	1.21	0			
	Metapopulation	0.59	0.97	0			
	Within Populations	0.49	1.06	0			

More complete results of all Scenarios are presented in Appendix 1.

** Notes:*

1. The variation in PE in the Tamarindo population among Scenarios 1-4 may well have been due to chance, rather than any true difference in the PE expected for these scenarios. The difficulty in estimating a more precise PE (even with 1500 iterations) is probably due to the great variance in population size seen in this r-selected species with intermittent breeding events. Production of offspring can vary greatly from year to year. Several bad years together can appreciably increase the probability of extinction in a wild subpopulation.
2. GD is not present in Scenario 5 because the way that dispersal between populations was modeled (as supplementation) artificially assumes that dispersing animals are unrelated to all existing animals. This elevates the reported GD above what would actually occur.

Summary of Modeling Results

The estimates of the population parameters provided by the experts on the PRCT lead to the conclusion that the current Tamarindo population is at a 3-4% risk of extinction over the next 100 years and will require continued human intervention to assure persistence. This intervention includes mitigation of the environmental problems and the augmentation of the subpopulation if necessary.

A metapopulation approach outline above for PRCT does decrease the probability of extinction over the next 100 years to zero. This is only due to the assumed persistence of the captive population. All wild populations continue to be vulnerable to extinction at ~3-4% rate if they are not directly supplemented by the captive population (Scenarios 2-4). As soon as augmentation ceases, the wild release populations are vulnerable to extinction, similar to what is projected for the Tamarindo population. It seemed to make little difference if this augmentation is for 10 or 20 years (Scenario 2 and 3).

All metapopulation scenarios substantially increased the amount of GD that is retained for the toad, from the 0.714 of initial levels seen for the Tamarindo subpopulation alone, to 0.899 when there are two release sites supplemented for 20 years (see Table above). This demonstrates a very positive benefit to a metapopulation approach for PRCT.

The current one-way dispersal of individuals as outlined in the first PRC Toad Metapopulation figure results in the need for continued human intervention to make sure that all populations persist. The problem is that all wild populations are vulnerable to extinction. If the toad does become extirpated from a site, there are no natural corridors to allow re-colonizing. Human intervention will be necessary if the population will again exist.

This current management plan was originally developed to prevent the spread of disease from one subpopulation to another. The subpopulations are completely isolated. While this model does afford some degree of disease transmission protection, it also increases the likelihood that the Tamarindo and release populations will not persist without natural or human re-colonization/augmentation. Consideration should be given to the development of wildlife corridors between subpopulations as outlined in Scenario 5. These could be natural, or by human translocation. The advantages to natural corridors are that they do not require translocation of animals into unfamiliar territory, which may significantly increase mortality. Natural migrating animals may have a much better survival rate. The disadvantages are that the natural migrating animals cannot be evaluated for disease and treated prophylactically to prevent pathogen transmission. If disease concerns are considered likely, translocation may be the best alternative if mortality rates are not too high.

Possible Future Modeling

The above modeling is an exploration of the stability of the PRCT populations. It gives an indication of the risks, which the metapopulation faces, and some of the management and research actions that might help to ensure its persistence. Accuracy of the model could be improved by determining the true carrying capacity, mortality rates, and fecundity rates of each wild population. Then, further analysis by the biologists and managers with responsibility for the population might be useful as further data are collected, additional management options are identified, or other questions about the viability of the population arise.

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Education and PR Working Group Report

Summary of Priority Actions

	Action	Priority / expected date of completion	Agency responsible	Contact
	Priority I: Increase Public awareness and profile in Puerto Rico			
1	Press release regarding meeting outcomes.	Week of Meetings	USFWS	Gloria Bell
2	Press release regarding mascot and the opening of the PRCT exhibit.	High / late January-February 2004	Juan Rivero Zoo and Toronto Zoo	Norma Villarrubia and Bob Johnson
3	Mascot costume completion.	High / early January 2004	Toronto Zoo	Bob Johnson
4	Utilize Jan Paul's photos or other photo for a flagship poster.	High / December 2003	SSP	Bob Johnson
5	Secure marketing professional to develop marketing campaign.	High / December 2003	Juan Rivero Zoo (Education coordinator)	Norma Villarrubia
	<i>(USFWS and SSP will serve as liaisons to the marketer.)</i>			
6	Solicit marketer to develop a logo, a slogan, products (souvenirs, toad models, t-shirts, caps), and solicit corporate sponsorships for target audiences and funding, place ads in local newspapers.	High / Beginning in December 2003. We need to develop a timeline for each aspect of this. Some is for the Feb-March blitz; some is for breeding seasons, some for is for festivals...	Toronto/Sedgwick Co./ Juan Rivero (Education coordinator)	Bob Johnson/Schane Anderson/Norma Villarrubia
7	Identify Puerto Rican education coordinator.	High / November 2003 (completed)	Juan Rivero Zoo	Norma Villarrubia volunteered

	<i>(Education coordinator will insure distribution of materials, identify local education advisors for material review and development)</i>			
8	Release updated educational materials. <i>Pages need to be photo-copy size and loose-leaf.</i>	High / January-February 2004	Toronto Zoo	Bob Johnson
9	Print existing pamphlet for Ernesto Estremera to distribute in the north.	High / January-February 2004	Toronto Zoo	Bob Johnson
10	Give materials to researchers for distribution in regional areas congruent with toad populations	High/ (timeline dependent on survey schedule).	Juan Rivero Zoo (Education coordinator)	Norma Villarrubia
11	Make games and puzzles similar to the ones at Juan Rivero Zoo.	med/January - February 2004	Juan Rivero Zoo	Daisy Rivera
12	Create pamphlet for Juan Rivero Zoo (modeled after existing pamphlet).	High / January-February 2004	Juan Rivero Zoo(Education coordinator) and Toronto Zoo/ Sedgwick County Zoo	Norma Villarrubia and Bob Johnson/Schane Anderson
13	Create activity book for grade 7 for Ernesto Estremera.	med/ Spring 2004	Toronto Zoo/ Sedgwick County Zoo	Bob Johnson/Schane Anderson
14	Liase with Citizens of the Karst to share education resources.	high/ Continual	USFWS and Juan Rivera Zoo(Education coordinator)	Silmarie Padron and Norma Villarrubia
15	Create student award for involvement in Sapo Concho Puertriqueno conservation education.	high/ late 2004-2005	(Education coordinator)	Ernesto Estremera and Norma Villarrubia

16	Develop generic materials for all of Puerto Rico.	med/ late 2004-2005	(Education coordinator) and SSP	Norma Villarrubia and SSP
17	Contract Ernesto's students for distribution of outreach materials.	high/ late 2004-2005		Ernesto Estremera and SSP
18	Identify audience that resources will go to -- generic vs. localized information.	med/ late 2004-2005	(Education coordinator)	Norma Villarrubia
19	Develop book on Sapo Cocho Puertorriqueno.	low/ late 2004-2005		Miguel Canals and Carlos Pacheco with university
20	Identify volunteers (i.e. high school and university students) for mascot, distribution of materials, monitoring.	high/ late 2004-2005		Ernesto Estremera, Norma Villarrubia, Daisy Rivera
21	Create information packets that will be based on the website information and available by mail upon request.	high/mid to late 2004	(Education coordinator)	Norma Villarrubia
	<i>packets will contain supportive information</i>			
	<i>How to get involved/volunteer</i>			
	<i>mascot</i>			
	<i>distribute materials</i>			
	<i>searches</i>			
	<i>lectures/interpreters at Guanica</i>			
	<i>Contact information</i>			
22	Have Mascot attend local festivals.	high/spring 2004 and beyond		
23	Follow up on short film that is in progress that can be used as a movie trailer.	med/ 2004		Miguel Canals

24	Create website.	high/mid to late 2004	Toronto Zoo will host (Web designer has been identified by Toronto)	Bob Johnson/Schane Anderson/Norma Villarrubia
	<i>website should have:</i>			
	<i>print friendly pages of activity book</i>			
	<i>email interaction with the public and be able to report toad sightings</i>			
	<i>audio toad calls</i>			
	<i>Sapo Concho Puertorriqueno Club</i>			
	Priority II: Develop local network of government, scientist, NGOs, etc.			
25	Set up listserv. Need to identify host and institute.	High/ December 2003	SSP/AZA or USFWS?	Can we nominate Diane Barber? I don't know if she will have time with the baby coming
	<i>(Concern was expressed that information not be released in ways or at times that could result in detrimental outcomes for the toads.)</i>			
	Priority III: Get more NGO's involved	December 2003 and ongoing	USFWS and SSP	
26	Conduct community meetings	med/ 2005?	SSP, Juan Rivera(Education coordinator)	Schane Anderson, Norma Villarrubia
27	Curriculum association 'education on wheels' project	med/ 2005?	SSP, Juan Rivera(Education coordinator)	Schane Anderson, Norma Villarrubia
28	Friends of the Karst	med/ 2005?	SSP, Juan Rivera(Education coordinator)	Schane Anderson, Norma Villarrubia

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**APPENDIX 1
Scenario Outputs
from *Vortex***

Appendix 1
Scenario Outputs from Vortex9.30

Scenario	#Runs	Population	stoc-r	SD(r)	PE	N-extant	SD(Next)	N-all	SD(Nall)	GeneDiv	SD(GD)	MeanTE
Base line K=1200	1500	Tamarindo	0.441	1.349	0.041	823.61	390.71	790.12	415.84	0.7141	0.1187	53.9
10% inc. mort. K=1000	1500	Tamarindo	0.324	1.393	0.136	759.75	410.39	656.43	461.92	0.6522	0.1451	47.5
K=1000	1500	Tamarindo	0.44	1.352	0.049	687.47	329.47	653.56	354.07	0.6683	0.1327	54.2
K=1400	1500	Tamarindo	0.442	1.348	0.035	983.49	453.39	948.74	480.92	0.7509	0.1013	54.5
No Catastrophes	1500	Tamarindo	0.521	1.297	0.004	882.32	358.86	878.79	362.45	0.763	0.0834	53.8
No F	1500	Tamarindo	0.452	1.362	0.035	825.16	396.27	796.01	417.97	0.7104	0.1194	48.7
4 lethals	1500	Tamarindo	0.433	1.337	0.044	841.04	387.08	804.03	415.94	0.7257	0.108	46
1) Meta Pop - 1 r-site Cont Intro	1500	Tamarindo	0.444	1.33	0.036	844.04	390.1	813.65	414.04	0.7288	0.1039	52.4
	1500	Captive	0.255	0.345	0	355.31	74.69	355.31	74.69	0.8173	0.0677	
	1500	Release	0.629	0.683	0	931.79	159.6	931.79	159.6	0.8139	0.0681	
	1500	Metapop	0.575	0.753	0	2100.75	504.3	2100.75	504.3	0.8802	0.0447	
	1500	WithinPop	0.443	0.786	0.012	710.38	208.13	710.38	208.13	0.7867	0.0799	52.4
2) Meta Pop - 1 r-site - 10 years	1500	Tamarindo	0.443	1.331	0.032	833.52	385.55	806.85	406.72	0.7255	0.1092	41.8
	1500	Captive	0.541	0.523	0	372.85	65.87	372.85	65.87	0.5954	0.1586	
	1500	Release	0.462	1.285	0.043	672.29	325.69	643.15	346.73	0.6986	0.1251	57.3
	1500	Metapop	0.59	0.94	0	1822.85	694.41	1822.85	694.41	0.8685	0.0651	
	1500	WithinPop	0.482	1.047	0.025	626.22	259.04	626.22	259.04	0.6732	0.131	49.5
3) Meta Pop - 1 r-site - 20 years	1500	Tamarindo	0.443	1.33	0.027	831.97	385.78	809.79	403.52	0.7239	0.1162	52.9
	1500	Captive	0.509	0.516	0	375.22	63.63	375.22	63.63	0.6128	0.1594	
	1500	Release	0.482	1.234	0.039	689.34	322.03	662.22	342.91	0.7082	0.1215	61.2
	1500	Metapop	0.588	0.924	0	1847.23	683.82	1847.23	683.82	0.8695	0.0635	
	1500	WithinPop	0.478	1.027	0.022	632.17	257.15	632.17	257.15	0.6816	0.1324	57.1
4) Meta Pop - 2 r-site - 20 years	1500	Tamarindo	0.443	1.33	0.029	823.51	388.64	799.9	406.94	0.7254	0.1092	43.5

	1500	Captive	0.51	0.514	0	372.6	64.96	372.6	64.96	0.6127	0.1556	
	1500	Release 1	0.467	1.254	0.027	683.14	324.89	664.93	338.91	0.7111	0.1188	61.3
	1500	Release 2	0.467	1.252	0.033	687.61	321.92	665.15	339.4	0.7126	0.1125	64
	1500	Metapop	0.578	0.984	0	2502.58	976.58	2502.58	976.58	0.8992	0.0477	
	1500	WithinPop	0.472	1.088	0.022	641.72	275.1	641.72	275.1	0.6904	0.124	56.2
5) Meta Pop – dispersal	1500	Tamarindo	0.463	1.292	0	862.39	370.61	862.39	370.61	0.9087	0.0297	
	1500	Captive	0.51	0.514	0	373.25	64.36	373.25	64.36	0.616	0.1534	
	1500	Release 1	0.487	1.213	0	711.44	307.51	711.44	307.51	0.907	0.0327	
	1500	Release 2	0.488	1.211	0	707.21	311.55	707.21	311.55	0.9043	0.0345	
	1500	Metapop	0.591	0.965	0	2654.29	915.88	2654.29	915.88	0.9583	0.0344	
	1500	WithinPop	0.487	1.058	0	663.57	263.51	663.57	263.51	0.834	0.0626	

Table Legend

Stoc. r	Mean population growth (r) in the stochastic simulations, calculated prior to any carrying capacity truncation, and averaged across years.
PE	Probability of extinction, assessed by the percent of simulated populations extinct by that year.
N-extant	Mean size of the simulated populations still extant at that year.
N-all	Mean size of all the simulated populations, including those that are extinct (with $N < 2$).
SD	Standard deviation across simulated populations.
GeneDiv	Gene diversity (expected heterozygosity) of extant populations, as a per cent of the initial gene diversity.
MeanTE	Mean time to extinction of those simulated populations that did go extinct.

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**APPENDIX 2
Participant List**

Puerto Rican Crested Toad PHVA ATTENDEES

Name	Organization	Address	Contact Information Tel/Email
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**APPENDIX 3
IUCN Policies and
Guidelines**

IUCN Policies and Guidelines

The following policy and guidelines of the IUCN-The World Conservation Union are relevant to the research and management of the Puerto Rican Crested Toad, and can be obtained from the websites listed below.

English language versions:

IUCN Red List Categories and Criteria

<http://www.iucn.org/themes/ssc/redlists/RLcats2001booklet.html>

Research Involving Species at Risk Of Extinction

<http://www.iucn.org/themes/ssc/pubs/policy/riske.htm>

IUCN Technical Guidelines on the Management of *Ex Situ* Populations for Conservation

<http://www.iucn.org/themes/ssc/pubs/policy/exsituen.htm>

IUCN Position Statement on Translocation of Living Organisms; Introductions, Reintroductions and Re-Stocking

<http://www.iucn.org/themes/ssc/pubs/policy/transe.htm>

Guidelines for Re-Introductions

<http://www.iucn.org/themes/ssc/pubs/policy/reinte.htm>

Versiones españolas:

Categorías y Criterios de la Lista Roja de la UICN

<http://www.iucn.org/themes/ssc/redlists/RLcats2001booklet.html>

Investigaciones en que se Usan Especies en Riesgo de Extincion

<http://www.iucn.org/themes/ssc/pubs/policy/risks.htm>

Directrices Técnicas de la UICN sobre la Gestión de Poblaciones *Ex Situ* para su Conservación

<http://www.iucn.org/themes/ssc/pubs/policy/exsitusp.htm>

Posición de la UICN con Respecto a los Desplazamientos de Organismos Vivos; Introducciones, Reintroducciones y Reconstitución de Poblaciones

<http://www.iucn.org/themes/ssc/pubs/policy/transs.htm>

Guías para Reintroducciones

<http://www.iucn.org/themes/ssc/pubs/policy/reints.htm>