

## **HUSBANDRY MANUAL**

### **PUERTO RICAN CRESTED TOAD (*Peltophryne lemur*)**

**2006/07 UPDATE**

Keeper and Curator Edition

Includes Veterinary Care Section and Shipping Instructions



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## TABLE OF CONTENTS

1. Introduction
  - 1.1. Description
  - 1.2. Habitat
  - 1.3. Wild Population
  - 1.4. Captive Population
2. Captive Management
  - 2.1. Releases
  - 2.2. Research
  - 2.3. PIT Tags
  - 2.4. Shipping
    - 2.4.1. Toad and toadlet shipping protocol
    - 2.4.2. Tadpole Shipping protocol
    - 2.4.3. Spanish Release Instructions
3. Exhibitory
4. Adult Husbandry
  - 4.1. Housing
  - 4.2. Disinfection
  - 4.3. Nutrition
5. Reproduction
  - 5.1. Sexing of Adults
  - 5.2. Pre-breeding Conditioning Period
  - 5.3. Breeding
  - 5.4. Hormonal Reproduction Protocol
6. Eggs, Tadpoles & Toadlets
  - 6.1. Egg Care
  - 6.2. Tadpoles
  - 6.3. Cleaning and feeding Protocol for Tadpoles
  - 6.4. Feeding Schedule
  - 6.5. Metamorphosis

7. Veterinary Care:

By Dr. Graham Crawshaw, Puerto Rican crested toad SSP Vet Advisor

7.1. Clinical Problems

7.2. Quarantine

7.3. Infectious Disease

7.3.1. Viruses

7.3.2. Bacteria

7.3.3. Fungi

7.3.4. Parasites

7.4. Non-infectious Disease

7.4.1. Nutritional Disease

7.4.2. Physical Factors

7.4.3. Toxins

7.5. Other Conditions

7.6. Mortality Peaks

7.7. Anaesthesia

7.8. Surgery

7.9. Blood Sampling and Haematology

7.10. Euthanasia

8. Products mentioned In the Text

9. References

10. Acknowledgements

Appendices

Tadpole Diet-Nutrient Composition	Appendix A
Tadpole Rearing Record	Appendix B
AZA Exhibit Survey By Elizabeth Bryant-Cavazos	Appendix C
Instructions for Domestic Shipments	Appendix D
Instructions for International Shipments	Appendix E
Report On Analysis of Algae And Cyanobacteria	Appendix F

This manual builds on "Captive Management of the Puerto Rican Crested Toad" by Dianne Devison of the Toronto Zoo published in 1995. It is intended as a guide for those working with this species and will be updated periodically as circumstances warrant.

## 1. INTRODUCTION

### 1.1. DESCRIPTION

Puerto Rican Crested Toads, *Peltophryne lemur* are the only native species of toad found in Puerto Rico and the Virgin Islands. This is a medium sized toad (64 to 120 mm snout-vent length) with distinctive supraorbital crests and a prominent upturned nose. Females are larger than males and have more prominent crests. Their colour ranges from brown to yellow brown with black or brown patches. The ventral surface is a creamy white with some dark mottling. Sexual dichromatism (males are yellower than females) is most obvious when males are in breeding condition. Males also possess prominent nuptial pads on the first and second digits of the front feet.

### 1.2. HABITAT

La Reserva Forestal de Guanica, the national forest in which Puerto Rican crested toads are still found, is a rugged dry sub-tropical forest. There are no permanent bodies of fresh water, and significant rainfall usually comes once a year. It is located on the south-west coast of Puerto Rico. The toads are found at low elevations (not exceeding 200m) in arid or semi-arid rocky areas with well drained soils. The habitat found in Guanica has an abundance of limestone ridges and boulders with dissolution holes and fissures, which are used by the toads as daytime retreats from the oppressive heat.



Daytime highs can reach 40 °C, and the surface temperature of the rocks can climb to 50 °C. A 1990 radiotracking inquiry into post reproductive movements showed that toads moved up to 1.5 km (0.9 miles) in 3 nights before settling down in an area where average nightly movements were 10 meters (33 feet). Toads were able to climb up vertical rock faces to seek shelter in holes in the limestone at least 45 cm (18 inches) above the ground.

### **1.3. WILD POPULATION**

Native to Puerto Rico and Virgin Gorda, this species is listed as threatened by the U.S. Fish and Wildlife Service. The Virgin Gorda population is thought to be extinct. Its range is currently limited to two known sites in Puerto Rico. These remaining populations, one found in the north at Quebradillas, and the other in the south at Guanica are known to be genetically distinct and must be managed as such.

### **1.4. CAPTIVE POPULATION**

As of 99.12.31 the captive population consisted of 84,501,26 animals (260) at 21 institutions. This includes 16 animals at University of Puerto Rico and ZooRico, Mayaguez. Wild caught founders number 20.5 at 2 Institutions. Of these, all are southern animals except the following: 3.1 Northern toads at Cincinnati (no founders), 3.0 Northern toads at Fort Worth (no founders.)

## 2. CAPTIVE MANAGEMENT ISSUES

### 2.1. RELEASES

Captive-bred animals have been designated for release in Puerto Rico, to supply the SSP with breeding stock, and for use in related research. Through the 1980s captive-bred offspring were held in captivity and returned to Puerto Rico post metamorphosis as toadlets or toads. Rearing animals post metamorphosis requires considerable resources (space, time, and food) and increases the shipping cost for a given number of animals. Now, captive-bred animals are sent to Puerto Rico at 7 to 10 days of age as tadpoles. This allows zoos to rear and return large numbers of animals for release without depleting often-scarce resources. Captive bred toads surplus to the SSP have been provided to the University of Puerto Rico at Rio Pedras and ZooRico, the zoo in Mayaguez. Staff and students at the university, and zoo staff have been trained and provided with the equipment necessary to hold and eventually breed the toads in Puerto Rico. The following is a history of the release of captive-bred Puerto Rican crested toads in Puerto Rico.

- 1982: First release of northern toads in Quebradillas 1982/06/19
- 1983: Buffalo releases: 75 toadlets released 83/5/23 Quebradillas (Northern)
- 1984: Buffalo releases: 500 toadlets released 84/4/29 Cambalache (Northern).  
306 toadlets released 84/6/21 Cambalache (Northern)
- 1985: Toronto releases: 370 toadlets released 85/12/2 Cambalache (Northern)
- 1987: Toronto releases: 829 toadlets released 87/6/23 Guanica (Southern)
- 1989: Toronto releases: 6.3 toads and 707 tadpoles (via Buffalo) released 89/02 Guanica
- 1991: Cincinnati releases: 352 toadlets (via Ft Worth) released Cambalache (Northern)
- 1992: Toronto releases: 1026 toads released in 92/12 Guanica (Southern)
- 1993: Toronto releases: 10,000 tadpoles released 1993 Guanica (Southern)  
Buffalo releases: 1,548 tadpoles released 1993 Guanica (Southern)
- 1996: Toronto releases: 1100 tadpoles released 96/05/07 Guanica (Southern)  
1000 tadpoles released 96/12/18 Guanica (Southern)
- 1999: Toronto releases: 1650 tadpoles released 99/04/18 Guanica (Southern).  
980 tadpoles released 99/11/23 Guanica (Southern).  
Sedgwick County releases: 2000 tadpoles released 99/04/18 Guanica (Southern).  
5000 tadpoles released 99/11/23 Guanica (Southern).  
Toledo releases: 4000-5000 tadpoles released 99/11/23 Guanica (Southern).
- 2000: Toronto releases: 25,000 tadpoles released 2000/12/03 Guanica (Southern)

## **2.2. RESEARCH**

Current research includes:

- The effects of temperature on tadpole growth and development.
- The effects of different diets on tadpole growth and development.
- The effects of different vitamin and mineral supplements on toadlet growth and development.
- The effects of different diets on the growth and development of toadlets.
- Substrate temperature selection by toads.
- Water temperature selection by tadpoles.

## **2.3. PIT TAGS**

At the Toronto Zoo, adult toads are implanted with passive integrated transponders (PIT). The Trovan PIT tags are used. Emla cream <sup>TM</sup> is used as a local anaesthetic, and is applied to the left shoulder area. The needle with the ID chip is inserted over the left thorax placing the implant subcutaneously between the left shoulder and the parotoid gland. The opening in the skin is closed with 3M Vetbond <sup>TM</sup> tissue glue. The area is then rinsed with water to remove traces of the anaesthetic, which is capable of causing general anaesthesia in these toads. Implanting with PIT tags means that all the toads can be housed together rather than in pairs, cutting down on holding space requirements and keeper time.

## **2.4. SHIPPING**

### **2.4.1. TOAD AND TOADLET SHIPPING PROTOCOL**

Toronto Zoo uses 1 or 2-litre wax coated cardboard milk cartons or large plastic deli/margarine containers for housing toads and toadlets for shipping. The containers must be thoroughly washed and rinsed and allowed to air dry before being used. Small holes (1/8" to 1/4") are punched or drilled in the sides and tops of the containers to provide needed air.

In order to provide moisture and a secure shock-absorbing environment in the shipping containers, slightly dampened sphagnum or sheet moss, teased or pulled apart to create air spaces and refuges for the toads is used as a packing medium. Each container is filled with this "airy" substrate. The moss must **not** be saturated with water. During shipping, wet moss will settle and the weight can crush, trap or drown small toadlets. Buffalo Zoo uses dampened paper towel to

provide the necessary moisture during shipping. Moistened sponge pieces or chips may also be suitable.

A one-litre container can hold twelve 1.25-cm (1/2") toadlets. The tops of the cartons are folded and taped or stapled closed. The square milk containers pack easily in Styrofoam shipping boxes. These are the same boxes that are used to ship tropical fish (a Styrofoam inner box placed in a cardboard outer box). To prevent jarring during transport crushed newspaper or Styrofoam packing chips are used to support the containers once in the boxes.

#### **2.4.2. TADPOLE SHIPPING PROTOCOL**

Tadpoles are packed and shipped in the same way that tropical fish are. Plastic bags (10" x 18") are used. These should be square bottomed or have the corners taped up on the outside to prevent tadpoles from being trapped in collapsed corners of bags. The bags are first filled with the tadpoles' original water to approximately 20% volume of the bag. Then an equal amount (20%) of aged or dechlorinated water (same temperature) is added to the bags for a total of 1.2 – 1.4 litres per bag. Approximately 300 tadpoles are put in each bag. To ensure adequate oxygen supply during shipping, the bags are then filled with oxygen from a medical cylinder to approximately 2/3 by volume of the bags. Bags are sealed using heavy rubber bands. The sealed bags are then placed into a second bag and tied again with heavy rubber bands. The shipping containers are the same ones used to ship tropical fish (a Styrofoam inner box placed in a cardboard outer box). The Styrofoam boxes are lined with several sheets of newspaper. To prevent jarring during transport, crushed newspaper or Styrofoam packing chips are used to support the bags once in the boxes. Another layer of newspaper is then placed over the bags. A can of Sera viformo tablet food (270 tablets per can) and a Spanish instruction sheet for releasing the tadpoles are included in each box. The Styrofoam boxes are taped up and placed in their cardboard outer shells, which are then also taped up.

The following shipping labels are then affixed to each box:

**-This way up**

**- Live animals**

**-Keep out of sun (in English and Spanish)**

Air transportation and shipping information can be found in Appendix D.



### **2.4.3. RELEASE INSTRUCTIONS**

#### **CORDIALES SALUDOS DESDE EL ZOOLOGICO DE TORONTO**

Antes de soltar los renacuajos en la laguna, es importante asegurarse que la temperatura del agua en la bolsa que contiene los renacuajos sea identica a la temperatura del agua en la laguna. Para ello proceda de la siguiente manera:

1) Desate la bolsa con los renacuajos y proceda lentamente a agregar agua de la laguna a la bolsa hasta que esta se llene de agua. Esta es necesario para aclimatar a los renacuajos a la temperatura y composicion quimica del agua del nuevo medio ambiente, evitando asi un posible "shock".

2) Una vez que los renacuajos se hayan aclimatado, se puede proceder a soltarlos en el agua.

Aqui les incluyo alimento para los primeros dias. Se puede alimentar entre 30 a 40 "pellets" por dia, dispersandolos en distintos puntos de la laguna. Tambien puede ofrecer algun vegetal verde como lechuga una vez al dia.

**BUENA SUERTE!!**

Greetings from the Toronto Zoo. Before releasing the tadpoles into the pond, ensure that the water temperature in the bags is the same as the pond. To accomplish this, undo the bags and slowly add the pond water to the tadpole bag until the bag is full of water. This is to acclimatise the tadpoles to the temperature and water chemistry of the pond. The tadpoles can then be released in the pond.

We have provided some tadpole food. Feed 30 to 40 pellets daily. Disperse throughout the pond. Lettuce or greens can also be fed once a day.

**GOOD LUCK!**

### **3. EXHIBITRY**

Some zoos have exhibited Puerto Rican crested toads using rock piles, or rock and cemented caves to simulate the limestone of the Puerto Rican habitat. These toads are a challenge to exhibit for they take every opportunity to hide and tuck-up in any crevice available. Substrates that have been used include sand, soil, moss, gunite, fibreglass, gravel, bark, river stone and limestone rocks. Shallow rock crevices and plants provide an environment in which the toads are usually visible, active and enjoyed by the public. A survey of AZA institution exhibits has been compiled by Eli Bryant-Cavazos and is attached as Appendix C. This survey describes exhibit set-ups and graphics.

#### 4. ADULT HUSBANDRY

##### 4.1. HOUSING (Off Exhibit)

At Toronto Zoo, Puerto Rican crested toads are housed off exhibit in 125 litre (35 gallon) aquaria, at a density of 6-10 adult toads per aquarium. If toads are not PIT tagged, animals that have bred are housed in pairs. The room the toads are housed in is well lit with natural lighting. To supplement natural photoperiod, a Vita-lite™ and Osram Sylvania Blacklight™ are suspended directly over the tanks on a 12:12 light: dark photoperiod. For ease of cleaning, the toads are kept on thin rubber matting in 125 litre (35 gallon) aquarium measuring 45cm wide x30cm high x90cm long (18" wide x 12" high x 36" long) with a plexi-glass and screen lid. Rocks with crevices, PVC tubes, coconut shells, and large leaves serve as hides. Toads commonly use 2" to 3" diameter PVC tubes (cut in 8" lengths) which can be linked with elbows and T's to form a network of tubes that can extend vertically as well as horizontally. The tubes are used as tunnels and daytime retreats. The network of PVC tubes can easily be disconnected for cleaning purposes and to locate a toad's whereabouts.

Water is provided in shallow dishes (plastic plant saucers) or by tilting the tanks and offering a small amount of water at one end (1-2 cm deep). Water sources are regularly utilised for defecation. Water dishes and tanks are cleaned daily by scrubbing soiled are and then rinsing and refilling with dechlorinated water.



Off exhibit holding tanks at  
Toronto Zoo

#### **4.2. DISINFECTION**

Disinfection is not routinely done but if required, a 1.0% sodium hypochlorite solution can be used. Household chlorine bleach ranges from 3% to 5% sodium hypochlorite and commercially available bulk solutions can be of much higher concentration (up to 12%). It is therefore vital to confirm the concentration on the container label or with the manufacturer or supplier before preparing a dilute working solution for use in empty tanks. After disinfection, tanks are rinsed thoroughly with hot tap water and left to air-dry overnight before being used to house toads again. Porous materials such as bark or driftwood are best replaced if excessively soiled. If these materials must be disinfected with bleach, they must then be rinsed with water and then sprayed with a solution of 16 ppm sodium thiosulphate (8mg per 500 ml of water). This solution has a neutralising effect on chlorine.

Iodine toxicity has been seen in amphibians from iodine reversibly bound to plastic holding containers. Because of this potential binding with plastics, it is recommended that iodine not be used to disinfect any material that will come in contact with the toads. Other disinfectants are not recommended for use on amphibian tanks or furniture.

### 4.3. NUTRITION

The adults are each fed one mouse pinkie every second week, and 3-4 large crickets (1 gram per toad) twice per week. All food is dusted with vitamin/mineral supplement. Occasionally mealworms, wax worms and dew worms are offered instead of the crickets. The toads are checked on feeding days and removed from their hides. This also ensures each toad gets its share of the food items. Some toads quickly adjust to the feeding regime and readily emerge from their hides on their own, anxious to feed. All the toads are weighed monthly in order to assess general health. They are not fed two weeks prior to and during the pre-breeding conditioning period (up to six weeks). After each breeding attempt, the toads are offered small amounts of food daily for about one week to increase lipid stores and then cut back to the two day per week feeding schedule.



Toads emerging from hides to feed.

## 5. REPRODUCTION

### 5.1. SEXING OF ADULT TOADS

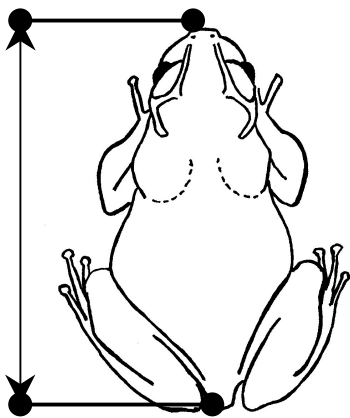
There are several prominent features that allow toads to be sexed as they approach adult size. The most reliable indicator is size. Measurements of adult toads are usually significant indicators of sex. Female toads are larger in size with a broader, more distinct headcrest (see table and diagram below).

**Table 1: Adult toad measurements**

	MALE	FEMALE
Weight range	27 to 54g	43-140g
Body length average	6.6cm	8.3cm
Body length range	6.1-7.5cm	8.1-8.4cm
Head length average	1.8cm	2.7cm
Head length range	1.8-1.9cm	2.2-2.9cm
Head width average	1.8cm	2.5cm
Head width range	1.8-2.0cm	2.5-2.6cm

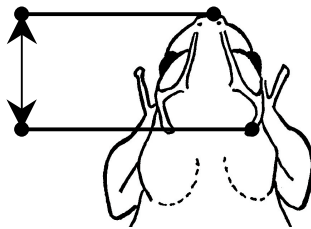
#### TOTAL LENGTH

Snout



Vent

#### HEAD LENGTH



Snout to middle of head at the end of cranial crest

#### HEAD WIDTH



Between the posterior edge of the cranial crests

When the males are in prime breeding condition, sexual dichromatism is particularly evident. Their colour changes to lemon yellow, particularly on the sides of the body, and the throat area. Males also possess prominent nuptial pads on the first and second digits of the front feet. Males will produce a male release call. By clasping the toad just behind its front legs, with thumb and

forefinger, it is possible to elicit a chirping release call from a male. This chirp may be barely audible or quite loud, depending on the toad, but in any case it is often possible to feel the jerking of the male's body as he exhales air to produce the call. This method is not infallible as some males do not readily produce the release call, and some females will attempt to do so.

## **5.2. PRE-BREEDING CONDITIONING PERIOD**

During the dry months in Puerto Rico, the toad becomes dormant and aestivates. This rest period may act as a stimulus for egg maturation in the ovaries of the females. The conditioning period prior to breeding Puerto Rican crested toads appears to be about 30-45 days. In the past, the pre-breeding conditioning involved aestivating toads in a moistened substrate of sphagnum and peat moss at a maintenance room temperature of 30°C. The substrate was allowed to slowly dry out, but it was found that the toads dehydrated as well. This method was discontinued due to toad deaths during and post aestivation.

Egg production and maturation in captive amphibians has often been unpredictable, possibly due to abnormalities in the vitellogenic cycle. Keeping amphibians at lower temperatures for 4 to 6 weeks has been shown to partially correct abnormalities in the vitellogenic cycle of captive amphibians. These animals produce larger numbers of mature ova that can support development. Through the limbic system, environmental cues such as temperature and light may affect the hypothalamus which secretes gonadotropin-releasing hormone (GnRH). GnRH causes the pituitary to release gonadotropin, which in turn stimulates steroid hormone production. The oestrogen steroid hormones play an important role in stimulating yolk protein synthesis in most of the vertebrates. The length of time and the degree of cooling, and the need to cool males, are currently under review.

Toads are maintained at a temperature range of 26°C-30°C (78°F- 86°F). As pre-breeding conditioning, Toronto Zoo now recommends that only female toads be cooled for 21-45 days. Before this conditioning period, toads are weighed and a general health examination is performed. If fat stores appear good and weight has remained stable, the selected toads are moved to a cool room, or incubator. In order to reduce the possibility of chytrid infection in Puerto Rican crested toads, the SSP is recommending a similar prophylactic treatment for fungal infections as that adopted by the Wyoming toad SSP. Prophylactic treatment of Puerto Rican crested toads consists of bathing the toads with Itraconazole (Sporanox, Janssen Pharmaceutica, Inc., Titusville, New Jersey, USA) prior to cooling. If Itraconazole is not available, Enilconazole

which is similar to itraconazole can be used. Enilconazole manufactured by Janssen Pharmaceuticals as Imaverol come as a 100mg/ml solution.

These recommendations are for all toads being bred with offspring destined for release to puerto rico, and does not apply to non-breeding toads. This protocol uses dilutions described by Donald K. Nichols and Elaine W. Lamirande of the Dept. of Pathology, Smithsonian Institute.

- A 0.01% suspension of itraconazole is used. This can be prepared from a 1% suspension of itraconazole (Sporanox, Janssen Pharmaceutica, Inc., Titusville, New Jersey, USA) using 0.6% saline as the dilutant.
- Toads are to be soaked in a depth of 2 cm of 0.01% itraconazole for 5 minutes daily for five days prior to cooling. The solution can be poured over the backs of the toads in order to wet the dorsal skin surfaces. The solution will be absorbed through the ventral skin and will be channeled to the entire toad.
- At the end of the cooling period and before placing the toads into the breeding tank, apply the 0.01% itraconazole as a rinse spray using a handheld spray bottle.

The female toads are not fed two weeks prior to and during the cooling period, but always have access to water. When a large number of toads are to be bred, the Toronto Zoo cools the room in which the toads are housed. For smaller numbers, the animals in their existing tanks, as is (i.e. no substrate other than the rubber matting and hides), are moved into an environmental chamber or incubator for cooling. The temperature is slowly dropped to 18°C(66°F) over the first 7 days of the cooling period. The open mesh of the aquarium lid is partly covered to raise the humidity to over 80% in the aquarium. As little disturbance as possible is encouraged during the cooling period.

In the past Buffalo Zoo has had successful breeding using the following pre-breeding conditioning. The toads are cooled in aquaria filled with wet sphagnum. The aquaria are moved to a cool room where temperatures range from 13-18°C(56-66°F), slightly cooler than at Toronto Zoo. The moss is kept generally wet throughout the cooling period, although the top layers are allowed to dry out somewhat. Temperature is either lowered or raised 5°F at a time, keeping the toads at a new temperature for 4-6 days until reaching the desired temperatures.

### **5.3. BREEDING**

Captive breeding events are scheduled for the late fall (Oct-Nov) to coincide with the likelihood of rain in Puerto Rico. If an important fall breeding fails, then an early spring (March-April)



breeding may be attempted. It is important to conduct a faecal examination after each breeding attempt. Despite normal loads, the stress of conditioning may affect the immune system response, and bacteria/parasite loads may be elevated.

Breeding tanks are set up with aged tap water well in advance (a minimum of 14 days) of the pre-breeding conditioning period begins. This allows 4-6 weeks for adequate preparation of filters and allows sufficient algae growth for tadpoles to feed on. These tanks are either 140L(35gal) aquaria or larger 400L(100gal) fibreglass tubs. The breeding tanks are set up on a slant with approximately 8cm(3") of aged or dechlorinated water at one end and a deeper end with 15cm(6") of water. This allows the adult toads the choice of water depth. The tanks are seeded with an algae culture (future tadpole food). The water temperature is maintained at 30°C(86°F). Large tubs have sponge filters and a canister filter circulating the water and the return is pumped through a perforated wand in an effort to simulate rain. The smaller aquaria have a sponge filter and a corner filter with charcoal.

A calcium block (primarily calcium carbonate), used in home aquariums, is also placed in the tanks. One such calcium block is the Wardley Turtle Tank Neutralizer with calcium (<http://www.bigalsonline.com/BigAlsUS/ctl3684/cp18505/si1382057/cl0/wardleysturtletankneutralizer>). Plastic plants, rocks and large half clay pots, some submerged, others half submerged to form out of water perching spots, are placed throughout the tank. Algae grows well on these and adults string their eggs around the clay shards and the plastic plants. In the wild, emergent plants are important as egg laying sites.

#### **5.4. HORMONAL REPRODUCTION PROTOCOL**

The following protocol used for the induction of reproduction in Puerto Rican crested toads at Toronto Zoo has proven successful on most occasions. The most success has occurred in young toads in their first breeding season, (males 1-2 years old and females 2-3 years old) however, hormone injections have stimulated egg laying in 7 year old toads at the Cincinnati Zoo, and 11 year old toads at the Toronto Zoo. The use of hormones for breeding amphibians does not negate proper pre-breeding conditioning. Hormone treatment will not be effective in the absence of mature ova in the ovary. In other anurans, ova maturation is dependent upon an extended maturation time and a good nutritional status. When selecting female for breeding, choose the most robust looking specimens. It is recommended that any females that are to be used for breeding be fed an extra pinkie mouse once a week for one month prior to the pre-breeding cooling period. Stress is a powerful inhibitor of amphibian reproduction and consequently needs to be reduced as much as possible.

Based on research conducted at Toronto zoo, two different hormones are used to induce reproduction. Luteinizing hormone releasing hormone (**LHRH**) **for females** and human chorionic gonadotropin (**hCG**) **for males**.

**LHRH for females:**

The analog des-Gly (d-Ala) LHRH ethylamide from Sigma Chemical (catalogue number L4513) is used in female toads. The product comes as a powder in 1 or 5 mg bottles, needs to be dissolved in sterile saline prior to use, and should be stored in the freezer. At TZ, LHRH is diluted to make a 100 microgram/ml solution and frozen in 1 ml syringes prior to use. The shelf life of the dry product is 2 years. Whenever possible, new product should be obtained for each breeding cycle. Toads are injected using a dose of 0.1 ug/g subcutaneously (intraperitoneal injection can also be used).

Sigma Chemical now requires USDA approval to sell LHRH for use in amphibians. Institutions should contact the USDA Center for Veterinary Medicine for assistance and submit an application to purchase LHRH for use in amphibians. To get permission for Sigma to sell LHRH to a zoo for use in animals each zoo needs to send a letter on Clinic's (Zoo's) letterhead to:

Kim Young (Tel: 1-240-276-9207; Fax: 1-240-276-9241)  
Deputy Director, Division of Compliance, HFV-230  
Center for Veterinary Medicine  
Food and Drug Administration  
Metro Park North  
7500 Standish Place  
Rockville Maryland 20855

Your veterinarian must submit the following information:

How did you learn of this product

Veterinarian's name, address and phone number.

Clinic name and address

Patient name and non-food species

Name of drug

Class of drug

Amount of drug

Condition to be treated with drug

A statement that you will notify the animal owner that the drug is not approved for use in animals; that the drug will not be used in food animals; that you agree to notify FDA if there are adverse reactions.

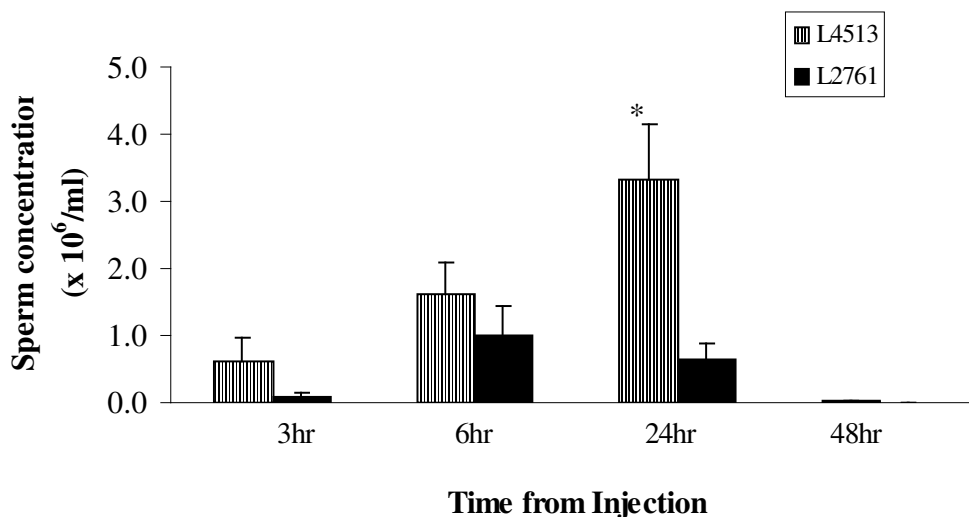
If the above contact information changes, updated information is available from their internet site (<http://www.fda.gov/cvm/default.html>) is:

U. S. Food and Drug Administration  
Center for Veterinary Medicine  
7519 Standish Place  
Rockville Maryland 20855-0001  
301-827-3800 or 1-888-INFO-FDA

### **hCG for males:**

Although there were no statistical differences in peak sperm concentrations in response to LHRH and hCG, we still recommend using hCG because of the timing of the spermiation response and the enhanced behavioural response seen. The timing of the peak appears to be sooner with hCG (3 to 6 hours) compared to LHRH (24 hours). The hCG dose that we recommend for males is 10 IU/g subcutaneously (intraperitoneal injection can also be used). Research shows that sperm production peaks between 6 and 24 hours following injection (See Graph 2).

**Graph 2: LHRH-induced spermiation in the Puerto Rican crested toad (125 ng/g dose)**



### **Timing of Injections:**

At the end of the cooling period the female toads are warmed back up to 28°C (82 °F) over three days (i.e. approx. 3 °C per day). On the third day taped toad calls\* are played throughout the day and into the night. As early as possible on the morning of day 3 the males are placed together in

the large breeding tank with simulated rain and the taped calls are played throughout the day. Between 11:00 and 13:00 hours, all the males are injected with hCG. Following the injection, the females are added to the breeding tanks. Wrestling of competing males appears to be very stimulating to male toads. As toads amplex they are moved to a separate breeding tank to keep track of parentage. Female toads are also injected with LHRH at this time. If toads are being bred only for release and parentage is not being tracked, the pairs can be left in the original breeding tank. Eggs are usually laid overnight within 24 hours of amplexus or after hormonal injections. Once eggs are laid, the adults are removed to holding tanks and the eggs are allowed to hatch in situ. The water level is then gradually increased in order to provide a larger volume of water for the developing eggs.

**If no eggs are laid overnight:** Females may be injected again the following day. Females seem to be susceptible to fluid overload and death from drowning can occur (see section **7.4.2 Non-infectious disease**). Caution should be used and the females' hydration levels and general body condition should be assessed at this time. If the females appear healthy and vigorous, then the toads can be re-injected and left in for an additional 24 hours.

**If pairs are not in amplexus:** Injecting females may cause premature deposition of unfertilised eggs. In these cases hormones can be re-administered following the same protocol and timing as above. Females are subsequently injected once a male has amplexed with them. Further injections are not usually required for males.

\*A cd of Puerto Rican toad calls is available from the Toronto Zoo.

## 6. EGGS , TADPOLES & TOADLETS

### 6.1. EGG CARE

After the adult pairs are removed from the breeding tanks, and detritus is siphoned out. Care is taken not to disturb the strings of eggs. Filters and return water are moved away from any eggs. Water depth is slowly increased until it reaches 18-23 cm (7-9"). Egg development is rapid at 30°C, and development is seen within 12 hours. After 24 hours the eggs hatch, and the embryonic tadpoles cling to the egg strings before dropping to the bottom of the tank. It is important not to disturb the embryonic and externally gilled tadpoles at this stage. The tanks are left another 24 hours and then any unhatched eggs are carefully siphoned out of the tank. Aeration by airstone or filter is kept low to avoid a whirlpool effect on the eggs, and newly hatched tadpoles. It is necessary to cover the filters with a fine netting (panty hose works well) to avoid trapping tadpoles inside the filter.

### 6.2. TADPOLES

**Recording hatch dates for toads:** The **hatch date** is the **date the tadpole emerges from the egg**. Metamorphosis dates can be recorded as separate comments. This is in accordance with the AZA "Standards for Data Entry and Maintenance of North American Zoo and Aquarium Animal Records Databases"

To maintain the highest water quality, detritus and uneaten food are siphoned off, and water changes with dechlorinated water (up to 40%) are done up to three times per day. Successful metamorphosis is more likely if the tadpoles are left in large volume tubs. When food items are placed in the tanks, a few tadpoles begin to feed, starting what appears to be a swarming around the food items. Tadpoles appear to move en masse throughout the tank.

The tadpoles are fed three times daily so that old food can be removed and fresh food is always available. Tank algae that consist mostly of blue green algae (605 *Leptolyngbya* cf. *boryana* sp) have been found to provide good nutrition. Algae should be cultured several weeks prior to breeding attempt so that there is an ample supply of fresh food available for tadpoles. Tank algae can also be harvested and stored frozen for future use. Please note that to avoid possible pathogen introduction, algae from ponds or other non-biosecure sources should not be used. Additional feed items include fresh high quality commercial fish foods. The morning feeding consists of equal parts Sera-san

enhancing flakes, Tetra 4 in 1 FD menu, aquaria herbivore diet, and spirulina flakes. The food is moistened with a few drops of water to form a firm ball and rolled in ascorbic acid powder. For example, 1/4 teaspoon (approx. 0.6 grams) of food plus supplement feeds 50 tadpoles (10 days old) at one feeding. Three to four feeding stations are provided per tank. The small balls of food are stuck to the tank wall just at the water surface, or are allowed to fall to the bottom of the tanks. The amounts and number of feeding stations are increased as the tadpoles grow. Calcium blocks will slowly dissolve in the water. A new one should be added to the water before the old one has completely dissolved.

Food from the morning feeding is removed in the afternoon prior to the second feeding. The afternoon feeding consists of Sera-viformo tablets. Two tablets (approx. 0.5 grams) are given per 50 tadpoles (at 10 days old).

The third feeding consists of pieces of 4" by 4" frozen or microwaved (to rupture the plant cells) spinach or lettuce per tank. Lettuce and spinach are fed on alternate days and the pieces are left in the tanks overnight. Previously frozen tank algae can also be used. This provides an overnight food source with little risk of fouling the water.

Prior to the morning feeding, the remaining food and faecal material from the previous night are removed. The pH, ammonia levels and the water temperatures are checked. Ammonia levels under 0.1 ppm, nitrates below 1ppm, pH between 7 and 8, and temperature of 30 °C(86°F) are recommended. Water changes to adjust water chemistry and to clean the water are performed. Water temperatures have ranged from 21-34°C (72-93°F) over the course of tadpole development. Recent studies at Toronto Zoo suggest that a temperature of 30°C (86°F) is best suited for raising healthy tadpoles. Ammonia and pH levels have ranged from 0.02 to 3.3 ppm and from 6.7 to 8.3 respectively, prior to morning water changes. Daily water changes vary from 10% to 50% depending on pH and ammonia levels and the clarity of the water. Sponge filters are rinsed clean with dechlorinated water once per week; filter wool and activated charcoal are changed three days later. Sudden fouling of the water due to tadpole mortalities or overfeeding can be addressed with massive water changes of up to 75% using fully aged or dechlorinated water.

Buffalo Zoo houses their Puerto Rican crested toad tadpoles in a glass box with a screen bottom. A 100% daily water change is performed by lifting out the tadpoles on the screen and placing them in a clean tank that has aged water at the same temperature. Water chemistry has not been monitored.

From studies done in Puerto Rico, metamorphosis occurs approximately 21 days after hatching. At cooler temperatures, captive metamorphosis can occur 10-14 days later than wild tadpoles. At 30°C, hind legs are obvious after day 9 and some tadpoles are ready to metamorphose by day 14. At this point "beach" areas are created in the tanks, by slowly dropping the water levels and offering more plant material and cork bark. From day 10 to day 21 (some tadpoles are delayed in development and some fail to metamorphose) front legs appear and toadlets start appearing on the "beaches".

### **6.3. CLEANING AND FEEDING PROTOCOL FOR TADPOLES**

#### A.M. Cleaning

1. Take water temperatures and record on individual enclosure sheets. Maintain temperature between 28-30°C.
2. Siphon off old food and faeces from bottom of tank. Remove up to 50% of water and replace with dechlorinated water at the same temperature.
3. Take water samples (prior to feeding) to lab on Monday, Wednesday, and Friday.
4. If ammonia levels are above 0.5 ppm do an additional 40% water change.
5. Every Friday - replace filter wool and activated carbon in all filters.  
Every Monday - rinse off sponge filters with dechlorinated water (a light rinse just to remove detritus on surface of sponge).

#### A.M. Feeding (after water changes)

1. Feed appropriate amounts of flake food mixture (see Appendix A for composition). Mix dry food with a small amount of water (2-3 drops) and roll into a ball. Roll ball in Vitamin C powder to coat and place at side of tank or drop to bottom of tank.

#### Mid-day Feeding

1. Siphon off uneaten food and faeces. Replace water with dechlorinated water at the same temperature. If indicated by water chemistry (ammonia levels above 0.5 ppm) do a 40% water change at this time.
2. Feed appropriate amounts of tablet food.

#### Evening feeding

1. Offer appropriate amount of frozen or microwaved lettuce or spinach.



#### **6.4. FEEDING SCHEDULE EXAMPLE**

The following amounts are averages fed to a tank of fifty tadpoles at 30°C at the specified ages (day after spawning).

##### A.M.: Flake Mixture

Day 2 - Day 5: 1/16 tsp. (0.15g)

Day 5 - Day 8: 1/8 tsp. (0.3g)

Day 8 - Day 14: 3/16 tsp. (0.45g)

Day 14+ - amount reduced as metamorphosis progressed.

##### Mid-day: Tablet Diet

Day 2 - Day 5: 3/4-tablet (0.18g)

Day 5 - Day 8: 1 1/4 tablet (0.30g)

Day 8 - Day 14: 1 1/2 tablets (0.38g)

Day 14+ - amount reduced as metamorphosis progressed.

Evening: Lettuce and Spinach:

Offered in 4" X 4" pieces ad lib (usually 2 to 4 pieces per tank) since it does not foul the water overnight.

#### **6.5. METAMORPHOSIS**

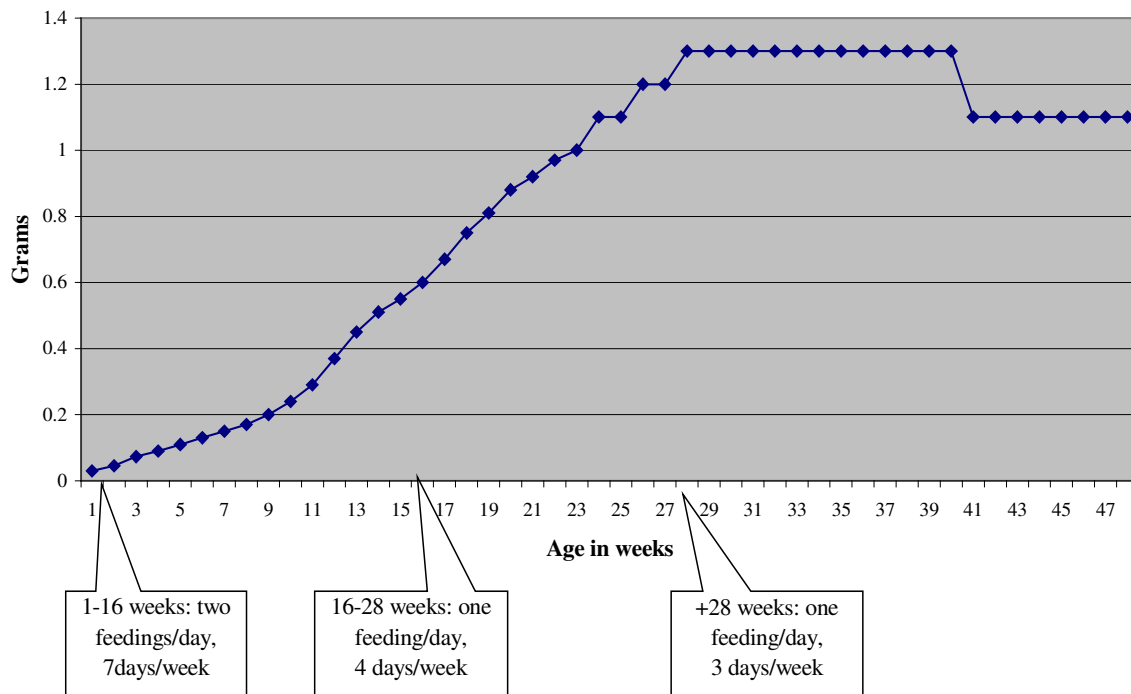
As toadlets start appearing on the "beaches", they are moved to toadlet rearing tanks. The Buffalo Zoo has had success using pea gravel as a substrate. Water is added to increase humidity and to prevent dehydration. At Toronto Zoo, the tank is set up with rubber matting or indoor/outdoor carpeting at one end of the tank and half coconut shells, clay shards, plastic hides, plastic plants and leaves are offered as refuges. One cm (.5 inch) of water is offered at the other end. To avoid desiccation after metamorphosing, the toadlets seek cover as soon as they leave the water. This

occurs even if water is available. Although many hide spots are offered, the toadlets usually mass together in large numbers under one or two hides. This may be an adaptive behaviour for moisture conservation since the surface area to mass ratio is much reduced. This drought reducing behaviour has been observed in both captive and wild situations with captive-bred toadlets.

The Toronto Zoo houses toadlets and toads in tanks with well fitted Plexiglas lids. Newly emerged toadlets are quite capable of scaling the vertical glass walls of these tanks, so secure lids are required. These lids have sections screened for air circulation. This also allows for some control over humidity levels, increasing or decreasing it by covering or exposing the screened areas of the tank lids. Since young toadlets are susceptible to dehydration, humidity in the tanks should be maintained over 80%.

The main food items for toadlets are pinhead crickets. If gravel is used as a substrate, care is required to ensure that the small crickets do not hide or disappear into the substrate. Fruit flies may be fed as well in the first few weeks. From Graph 1 below, the appropriate amount of food to offer for a group of toadlets can be determined. For example, a group of 25 toadlets at 5 weeks would require 2.5grams of food.

**Graph 1: Average weight of food offered per Toad**



Young toadlets have a very robust appetite and have been known to overeat. To prevent deaths from ingesting too large a meal, the daily ration per toad is divided in half and the young toads are fed twice a day, every day, for the first 14-16 weeks. From age 4 to 6 months they are fed the full

ration during a single feeding offered four times a week. After six months of age they are feed three times a week on the same schedule as the adults. As the toadlets increase in size, so does the cricket size. At eight to ten weeks they are offered 1/4" crickets, at 16 weeks they are offered 1/2" crickets and at 24 weeks they are offered 3/4" adult crickets, wax worms and mealworms. As they approach adult size, other food items such as dew worms or new-born mouse pinkies are introduced. At this time they are put on the same feeding schedule as adults, and the amount of crickets offered is slightly reduced to 1-1.2 grams per adult toad.

## **7. VETERINARY CARE**

**By Dr. Graham Crawshaw, Puerto Rican crested toad SSP Vet Advisor**

### **7.1. CLINICAL PROBLEMS**

When provided with the appropriate living conditions, the Puerto Rican crested toad has proven to be a resilient and long-lived animal in captivity. Disease conditions have been seen in individuals as well as in groups. There is no reason to believe that the Puerto Rican crested toad is in any way unique among amphibians from a disease point of view, but there is still little documented evidence of any specific disease processes. Most deaths have been sporadic, although multiple die-offs have occurred in both adults and juveniles. As with other amphibians, the diagnosis of the extent and cause of a particular problem in the live animal is not easy. A complete necropsy may be required to elucidate the problem and develop a course of treatment for other affected individuals.

### **7.2. QUARANTINE**

A suitable quarantine period in order to evaluate in-coming animals, to allow them to adjust to new conditions, recover from transit and to avoid transfer of disease to an existing collection. At Toronto Zoo, Puerto Rican crested toads are maintained in quarantine rooms for a minimum of 60 days or until animals are considered healthy, free from parasites, eating well and are gaining weight. The quarantine will be extended indefinitely if these conditions are not met. Animals are weighed upon arrival and a brief examination is performed. Later, at some stage during the quarantine period closer examination, identification, and parasite treatment are performed. Antibiotics are not used routinely unless the condition of the animals or medical history dictates. At least three negative faecal samples are required before animals are released from quarantine. Since Puerto Rican crested toads or their offspring may be designated for transfer to other institutions or for release in to the wild, protection of the species from medical conditions which may be present in other amphibians in the collection is highly desirable. The use of a separate room or facility for Puerto Rican crested toads is strongly encouraged.

### 7.3. INFECTIOUS DISEASE

#### 7.3.1. VIRUSES

Viral disease is as of yet undiagnosed in Puerto Rican crested toads. There have been cases of high tank mortality in groups of tadpoles that have generalised oedema reminiscent of lesions caused by the iridovirus, tadpole oedema virus, but no attempts have been made to isolate a virus.

#### 7.3.2. BACTERIA

As with other amphibian species, bacterial disease is not uncommon. Many of the cases of sepsis have been associated with outbreak situations during times of stress, such as aestivation, introduction to breeding tanks, or in young individuals. Post-shipment mortality has also been seen. Under normal circumstances conditioned established adults appear to be very hardy. While every effort should be made to keep the toads' environment free from pathogens, over cleaning, excessive disturbance and the use of disinfectants can be stressful and may render toads more susceptible to infectious disease. Affected animals tend to be lethargic and less responsive than normal, fluid accumulation may be seen in the legs and coelomic cavity, and reddening of the ventral surface and of the toes may be apparent. Occasional skin ulcers may be seen particular on the feet and at the points of contact with the ground. As with all amphibian species, bacterial septicaemia and dermatitis are of concern in Puerto Rican crested toads. Most of the bacterial species of concern are gram-negative opportunistic invaders. Organisms isolated from cases of sepsis and dermatitis include *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Bordetella*, *Alcaligenes*, and *Serratia*. Multiple organ systems are often infected in cases of septicaemia, including liver, kidney, spleen, and occasionally heart. *Acinetobacter* has been isolated from a case of pneumonia.

Thirty eight cases of systemic disease have been seen at Toronto Zoo . Most of these were diagnosed with single or multiple organism septicaemia. *Pseudomonas* spp. were the most commonly isolated bacteria from septic animals. *Flavobacterium*, *Bordetella*, *Alcaligenes*, and *Serratia marcescens* have also been cultured. In only four individuals was *Aeromonas hydrophila* cultured. There has been very little need for the use of antibiotics in Puerto Rican crested toads, since under Toronto Zoo conditions either individual or group outbreaks of bacterial infection are uncommon. Antibiotics of choice include aminoglycosides (Amiglyde, 5mg/kg s.c. q24h) and fluoroquinolones (Baytril-enrofloxacin, 5-10 mg/kg p.o., or s.c. q24h). Tadpoles also get bacterial sepsis, often associated with poor water quality or some other stress. Treatment of tadpoles can be performed with methylene blue baths (3 mg/l), or antibiotics.

Mycobacteriosis (tuberculosis) is a common condition in captive amphibians, and is manifested by either skin lesions (nodules and ulcers) or generalised disease. No records of mycobacteriosis causing death of Puerto Rican crested toads exists, but acid-fast bacteria has been identified in an abscess of a live toad.

### **7.3.3. FUNGI**

Fungal dermatitis and systemic fungal disease are occasionally seen in the clinical setting. *Nigrosporum* and *Trichoderma* have been isolated in fungal dermatitis at Toronto Zoo. Lesions are variably distributed, and typically black, necrotic and ulcerated with occasional white raised areas. In addition, one case of dermatitis has been seen in which the skin was black, necrotic and oedematous. Tadpoles occasionally also get fungal infections manifesting as multiple deaths within a tank with fungal organisms evident grossly or histologically. One case of chromoblastomycosis, a systemic fungal disease that has been well documented in other amphibian species has also been seen. Treatment of fungal disease can be undertaken with topical or systemic therapy. Itraconazole (Sporanox™)(10 mg/kg p.o. q 24h) or ketoconazole (10-20 mg/kg PO q12-24h) has been utilised with some success in amphibians. Topical therapy with enilconazole (Imaverol™) suspension was successful in treating the case of *Trichoderma*. While chytridiomycosis has not been documented in Puerto Rican crested toads it has been diagnosed in other bufonids, and there is no apparent reason the species should not be considered susceptible to infection by chytrids. Lesions are described as having a predominantly ventral distribution with a brown discoloration and granular appearance. Animals are often found dead before gross lesions are noted. No treatment for chytridiomycosis has been described.

### **7.3.4. PARASITES**

Parasitic disease has been relatively uncommon in captive Puerto Rican crested toads. Strongyloid infestation has been noted in a few individuals and has been quite refractory to treatment with various anti-parasitic drugs. A group of individuals at one zoo was diagnosed histopathologically with a *Balantidium*-like enteric parasitism. As there was no gross evidence of enteric disease or histologic evidence of inflammation, the pathogenesis remains unclear. Amoebic enteritis was diagnosed in one individual. *Entamoeba* has been shown to be pathogenic in other amphibian species, and may be diagnosed on direct smears or flotation, although systemic infection of the liver and kidneys can also occur. One zoo reported an infection with a coccidian. These are traditionally hard to eliminate in amphibians and reptiles.

Treatment of parasitic diseases has met with variable success, depending on the parasite. Nematodes can be hard to eliminate permanently either due to ineffectiveness of the treatment or reinfestation from the environment. At Toronto Zoo the current treatment for strongyloids is with

moxidectin (Cydectin™) (1mg/kg s.c., q14d). Fenbendazole (Panacur™)(100 mg/kg p.o. q14d), and ivermectin (Ivomec™) (0.2-0.4 mg/kg s.c, q14d) have also been used.

Flagellates are usually not of concern, but when present in large numbers can cause weight loss and the faeces will be liquid brown. These have been successfully treated with Flagyl (Metronidazole) 100mg/kg mixed with 1 ml Hill's Prescription Diet a/d with 0.5ml water PO Q3D for 5 treatments. If the faeces is very loose and an enteritis is suspect we will add Baytril 10mg/kg to the mix.

## **7.4. NON-INFECTIOUS DISEASE**

### **7.4.1. NUTRITIONAL DISEASE**

Nutritional requirements of Puerto Rican crested toads, while being specifically undefined, seem to be generally met by the captive diets used at many zoos. Metabolic bone disease either associated with hypocalcemia or hypovitaminosis D has been seen in growing toadlets which have very high growth rates. In addition, adults may develop fibrous osteodystrophy, pathologic fractures and vertebral malformation secondary to long term calcium or vitamin D deficiencies. The use of powdered supplements and high calcium invertebrate diets are essential when feeding insects that often have inverse calcium: phosphorus ratios and are deficient in certain other nutrients.

Spindly leg syndrome has been seen in Puerto Rican crested toads, and as in other species, the cause has not been determined. The condition may affect up to 100% of developing toadlets but it appears to be more common in offspring from older breeding stock. Tadpoles appear to develop normally until the time of limb generation, at which time very small, poorly muscled forelimbs develop. At this stage no treatment is feasible and although affected animals may survive for some time, euthanasia is recommended. The cause of this disease also remains undefined and multiple factors may be involved. With the diet fed at the Toronto Zoo, for which nutritional analysis has been performed, this syndrome is not seen commonly. It is vitally important that the ingredients for the tadpole diet, including vitamins are fresh. Oxidation of some of the nutrients is likely a contributing factor in spindly leg syndrome.

Other developmental deformities have also been seen in juvenile Puerto Rican crested toads including missing eyes and rotated elbows. A cause has not been determined. Gastric impaction secondary to overfeeding has been seen in a few Puerto Rican crested toads, and consideration should be given not to overfeed individuals. Young and small animals may be at higher risk due to the lower potential gastric volume.

Myopathy (muscle pathology) has been seen in a number of individuals. While no aetiology has been defined for this myopathy, nutritional deficiencies (i.e. hypovitaminosis E) are a concern,

and vitamin E levels should be quantified in suspect cases. A hind limb paralysis has also been seen in a number of individuals. Whether or not nutritional deficiencies (i.e. vitamin E, A, or B) may play a role in this disease is not known.



#### **7.4.2. PHYSICAL FACTORS**

Drowning is seen periodically in individuals at breeding time. Small females, that cannot support the weight of amplexing males, are particularly at risk. Submerged structures where individuals can sit at the water surface are helpful in preventing drowning, but not totally effective. Metamorphosing toadlets may drown if left in deep water too long without access to shoreline once their forelimbs emerge. Trauma is an occasional and unfortunate cause of death. Mortality has been associated with preparation for breeding both through drying (aestivation) and cooling (hibernation). Accidental excessive cooling has lead to deaths in hibernated breeders. As with any amphibian species, *P. lemur* is susceptible to desiccation particularly in small individuals. Animals aestivated in drying conditions may also succumb to desiccation if the substrate is too dry. Animals aestivated in this manner must be monitored carefully for dehydration. Mortalities have also been seen when cooled or aestivated animals are placed in breeding tanks and rehydrated. It is not uncommon that individuals put into breeding tanks 24-48 hours after being removed from aestivation die from overhydration with lesions of ascites (coelomic fluid) and generalised oedema. Older animals appear to be much more susceptible. It is suggested that these animals be rehydrated more slowly over a period of 2-3 days before being placed into breeding tanks to allow for a more gradual return of normal osmoregulatory function, and that they be monitored closely. It is possible that the hormone used to induce reproduction (luteinizing hormone releasing hormone) may increase susceptibility to osmoregulatory imbalances since the hormones associated with egg-laying oxytocin and arginine vasotocin increase the permeability of skin. To counteract this effect, furosemide (Lasix <sup>TM</sup>) may be given by injection (5mg/kg s.c.).

#### **7.4.3. TOXINS**

As with all amphibians, the Puerto Rican crested toad is susceptible to environmental contamination and toxicity. Tadpoles and toadlets are the highest risk groups. Degradation of environmental conditions and contamination with such things as cleaning chemicals should be considered in cases of high tank mortalities. Regular testing of water for ammonia, nitrates, pH, and temperature is imperative. Ammonia levels under 0.1 ppm, nitrates below 1ppm, pH between 7 and 8, and temperature between 29 °C and 31 °C are recommended. Regular water changes or excellent biological filtration are generally recommended to maintain such conditions. High levels of dissolved gases can cause "gas bubble disease," in tadpoles as is seen in fish.

#### **7.5. OTHER CONDITIONS**

Tumours are generally quite rare in amphibians. One case of myeloproliferative disease was diagnosed at necropsy. Kidney and urinary pathology is common in older animals. The majority

have glomerular disease. A cluster of four cases of cystic nephropathy was noted at one zoo. The causes remain unclear.

## **7.6. MORTALITY PEAKS**

Mortality peaks are associated with particular times in the life cycle of captive Puerto Rican crested toads. Infertility does occur. It is not uncommon that 15-25% of a clutch of eggs is infertile. Higher levels of infertility may also be seen. While tadpole survivability is greatly increased in captivity, the tadpole stage can experience high mortality. Tadpole mortality rates seem to peak within the first few days after hatching and at or near metamorphosis. However, large numbers of tadpole deaths should not be considered normal and diagnostic efforts should be pursued. Tadpoles that are fed adequately grow at variable rates, and a small percentage of individuals never seem to flourish. The exact reasons for this are unclear, and undoubtedly these are individuals that would not survive natural conditions.

## **7.7. ANAESTHESIA**

Anaesthesia in Puerto Rican crested toads is similar to anaesthesia of other toad species. For general anaesthesia toads are placed in a container containing a shallow depth of water containing 2 g/l tricaine methanesulphonate (MS-222). Solutions should be buffered to bring the pH close to neutral using the same concentration (2g/l) of sodium bicarbonate (powder or premixed solution). The toad sits in and is soaked in the solution until the appropriate level of anaesthesia is achieved. This can be judged by increasing muscular relaxation, loss of righting reflex, lack of response to pain, and slowing respiration. Induction usually takes 8-12 minutes at room temperature (smaller animals induce faster).

Blood collection and surgery can be performed following MS-222 anaesthesia. At surgical levels, respiration ceases. Toads have been kept anaesthetised for an hour or more (especially at cool temperatures) without the need for additional anaesthetic. In fact, keeping the animals in anaesthetic solution will result in overdose and death. Additional MS-222 can be applied or injected if needed to maintain surgical plane. Animals should be kept moist during surgery. Following completion of the procedure, toads are rinsed in clean water and allowed to recover. It is recommended that toads are warmed to reduce recovery times – this can be achieved by placing the container on a heating pad.

Local anaesthesia is used for placing transponders and surgery of superficial lesions. Emla cream <sup>TM</sup> is applied to the location with cotton tipped applicator and rubbed in. In toads anaesthesia of the area is achieved within a few minutes. Avoid excessive application of the cream as this can cause general anaesthesia. Excess cream is wiped and rinsed of at the end of the procedure.

## 7.8. SURGERY

Puerto Rican crested toads are quite amenable to surgery if satisfactory anaesthesia can be achieved. Laparotomy, laparoscopy and skin surgery can be performed. Local anaesthesia can be used for superficial biopsies and lacerations. Wounds are sutured with a soft absorbable suture (e.g. Vicryl <sup>TM</sup>) and VetBond <sup>TM</sup> glue is applied to the suture line. For small wounds, VetBond alone is used.

## 7.9. BLOOD SAMPLING AND HAEMATOLOGY

The usefulness of blood samples in the diagnosis of amphibian disease is much less proven than in other taxa. Blood parameters are more labile in amphibians and there has been little work on correlating changes in blood values with disease. However, sampling is encouraged in order to develop knowledge in this area. Obtaining samples can be difficult and cardiac puncture remains the most reliable source for blood. Anaesthesia is recommended, to achieve deflation of the lungs and allow palpation of the heart. Puerto Rican crested toads typically have high basophil counts - a feature common to some amphibians.

A study at the Toronto Zoo in 1998 looked at haematological parameters. Blood samples were taken from anaesthetised toads by cardiac puncture. The heart is located beneath the sternum (it may be necessary to retract the xiphoid process cranially). Blood is collected via a small gauge needle and syringe introduced into the ventricle with gentle aspiration. This procedure appears to be quite safe. Haematological parameters of forty (eight-month-old) anaesthetised toads are presented in Table 2.

**Table 2: Haematological parameters**

Parameter	Mean	Standard Deviation
Erythrocyte count (automated) ( $\times 10^{12}/l$ )	0.987	0.109
Erythrocyte count (manual) ( $\times 10^{12}/l$ )	1.237	0.208
Leukocyte count (manual) ( $\times 10^9/l$ )	10.16	3.071

Parameter	Mean	Standard Deviation
Neutrophil (%)	4.400	2.458
Lymphocyte (%)	63.76	8.224
Basophil (%)	27.73	7.225
Eosinophil (%)	3.000	3.242
Monocyte (%)	1.325	1.421
Hematocrit (%)	32.93	3.744
Hemoglobin (g/l)	105.2	13.90
Protein (total) (g/l)	38.45	5.5
Albumin (g/l)	18.00	2.699
Albumin/Globulin ratio	0.885	0.158
Alkaline Phosphatase (IU/l)	51.90	19.36
Alanine Aminotransferase (IU/l)	13.68	7.956
Aspartate Aminotransferase (IU/l)	228.5	154.5
Bile Acids (total) (μmol/l)	1.780	0.645
Bile Acids (Conjugated) (μmol/l)	0.480	0.304
Amylase (IU/l)	91.73	80.98
Lipase (IU/l)	53.73	20.63
Calcium (mmol/l)	2.015	0.210
Chloride (mmol/l)	76.18	6.520
Phosphorus (mmol/l)	1.863	0.673
Potassium (mmol/l)	4.30	1.48
Sodium (mmol/l)	109.5	5.849
Sodium/Potassium ratio	30.43	15.94
Creatinine Phosphokinase (IU/l)	2389	1852
Creatinine (μmol/l)	28.23	5.568
Urea (mmol/l)	15.04	6.437
Glucose (mmol/l)	2.720	0.958

#### 7.10. EUTHANASIA

Tadpoles and small toadlets (under 5 grams) may be euthanised by placing them in a solution of T-61 or pentobarbitone. Large toads should be injected individually (i.p.).

## 8. PRODUCTS MENTIONED IN THE TEXT

- Amikacin (Amiglyde™) – Ayerst Veterinary Labs, Wyeth-Ayerst Canada Inc. 400 Michener Rd, Guelph, ON N1K 1E4, Canada
- Ascorbic Acid, coated - Hoffman-LaRoche Ltd., Etobicoke, Ontario, M9C 5J4, Canada.
- Aquarian Herbivor Diet –
- Enrofloxacin (Baytril™)—Bayer Inc. Ag. Division- Animal Health, 77 Belfield Rd. Toronto, Ont. M9W 1G6
- Calcium blocks - Turtle Tank Neutralizer: Wordley Corporation, New Jersey, U.S.A.
- Enilconazole (Imaverol™) Janssen Animal Health, 19 Green Belt Dr., Toronto, ON M3C 1L9, Canada
- Fenbendazole (Panacur) – Hoechst Roussel Vet, 240 Henderson Dr., Regina, SK, S4N 5P7, Canada
- Furosemide (Lasix™) - Hoechst Roussel Vet Vet, 240 Henderson Dr., Regina, SK, S4N 5P7, Canada
- Hill's Prescription Diet a/d – Hill's Pet Nutrition, Inc. P.O. Box 148, Topeka Kansas, [www.hillspet.com](http://www.hillspet.com)
- Itraconazole (Sporanox™)Janssen-Ortho
- Ivermectin (Ivomec™) – Merial Canada Inc., 500 Morgan Blvd. Suite#1, Baie D'Urfe, PQ H9X 3V1, Canada
- Luteinizing hormone releasing hormone analog des-Gly (d-Ala) LHRH ethylamide: Sigma Chemical (catalog number L4513)
- Metronidazole (Flagyl)
- Moxidectin (Cydectin™), Ayerst Veterinary Labs, Wyeth-Ayerst Canada Inc. 400 Michener Rd, Guelph, ON N1K 1E4, Canada
- MS222™- Sandoz, Basle, Switzerland
- Murex Spirulina Flakes - Murex Aqua Foods Inc., Langley, British Colombia, V3A 5E8, Canada.
- Osram Sylvania Blacklight F40/350BL-40W—Osram Sylvania, 100 Endicott St. Danvers, MA, 01923, USA
- Sera San Colour Enhancing Flakes - Sera Aquarisk GmbH 5138 Heinsberg, Germany.
- Tetra FD Menu, Special Food, 4 in 1 blend: Tetra Werke, Ulrich, Germany
- Trovan™ – InfoPet Identification Systems Inc., 517 W. Travelers Trail, Burnsville, MN 55537-2548, USA
- Vatbond™ – 3M Animal Care Products, St. Paul, MN 55144-1000, USA
- Vita-lite™, Duro-Test Corp. 9 Law Dr., Fairfield, NJ 07004, USA



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## 10. ACKNOWLEDGEMENTS

Dianne Devison, Supervisor Toronto Zoo; Rick Paine, Buffalo Zoo; Cincinnati Zoo; Dr. Graham Crawshaw, Veterinarian, Toronto Zoo; Nubar Dakessian, Keeper, Toronto Zoo; Elaine Gabura, Puerto Rican crested toads Studbook keeper, Toronto Zoo; Bob Johnson, Curator Amphibians and Reptiles Toronto Zoo; Mavis Russell, Animal Care Clerk, Toronto Zoo; Eldon Smith, Curatorial Assistant, Toronto Zoo; Dr. Eduardo Valdes and Nutrition Centre staff, Toronto Zoo; Animal Health Centre staff, Toronto Zoo;

**Appendix A                      TADPOLE DIET NUTRIENT COMPOSITION**

<b>Component</b>		<b>Dry Basis</b>	<b>As Fed</b>
Dry Matter	%	100.00	93.00
Crude Protein	%	51.18	47.60
Crude Fat	%	5.81	5.40
Ash	%	12.04	11.20
Crude Fibre	%	1.83	1.70
Gross Energy	Cal/g	4.920	4.575
ADF	%	2.47	2.30
Ca	%	2.47	2.30
P	%	1.45	1.35
Mg	%	0.17	0.16
K	%	0.88	0.82
Na	%	0.77	0.72
Fe	Ppm	1018.82	947.50
Zn	ppm	142.69	132.70
Cu	ppm	6.88	6.40
Vit C	mg/g	8.67	8.11
Vit E	mg/kg	396.77	369.00
Vit A	IU/kg	5836.70	5428.13
Vit D	IU/g	3.33	3.10
Asp	%	4.15	3.86
Glu	%	7.28	6.74
Ser	%	2.18	2.03
Gly	%	4.37	4.06
His	%	1.12	1.04
Arg	%	2.90	2.70
Thr	%	1.97	1.83
Ala	%	3.17	2.95
Pro	%	3.22	2.99
Tyr	%	1.42	1.32

**Appendix A (cont.)      TADPOLE DIET NUTRIENT COMPOSITION**

<b>Component</b>		<b>Dry Basis</b>	<b>As Fed</b>
Val	%	2.20	2.05
Met	%	1.22	1.13
Ile	%	1.81	1.68
Leu	%	3.40	3.16
Phe	%	1.95	1.81
Cys	%	0.44	0.41
Lys	%	3.12	2.90

Flake diet Ingredient Composition

Aquarian Herbivore Diet	100g
Tetra FD-Menu 4 In 1 Blend	100g
Sera-San Color Enhancing Flakes	100g
Murex Spirulina Flakes	100g
Ascorbic Acid, Coated	2g
Jameson B50	1 tablet
Thiamine	100mg

## TADPOLE REARING RECORD

Month:                      Year:

Species: Puerto Rican Crested Toad

I.D.:     Dam: \_\_\_\_\_

I.D.:     Sire: \_\_\_\_\_

Date Laid:

Notes: Follow posted Protocol

[illegible]

**Appendix C**

**AZA EXHIBIT SURVEY BY ELIZABETH BRYANT-CAVAZOS**

**Part 1: Graphics and Exhibit Technique**

INSTITUTION	GRAPHICS	SECRET TO EXHIBITING TOADS
Buffalo	We currently have a very basic graphics: new graphics would highlight conservation (particularly the release program) and adaptations of semi-fossorial amphibians in harsh environments.	They are not capable of burrowing into the hard mud (the dried mud looks great to) and the toads hang out in the stone bunkers in view of the public. They get active for feeding and misting.
Calgary	Habitat loss is the major reason why these distinctive looking toads are endangered. Zoos are trying to help Puerto Rican crested toad populations recover through the Species Survival Plan breeding and release program.	Haven't been able to keep visible. Possibly try small potted plants at front of the exhibit. They seem to like sitting in the pots under vegetation
Cleveland Metroparks	The sensitive skin of frogs and toads is highly permeable, allowing water and air to pass, much like out lungs. This unique property also makes the animals particularly vulnerable to pollutants and pesticides. Amphibians act as environmental monitors, providing us with early warnings of changes in the Rainforests conditions. At the bottom of the graphic -- SSP The Puerto Rican crested toad is highly endangered due to extensive habitat destruction on its tropical island. Cleveland Metroparks Zoo is an active participant in an international effort called Species Survival Plan (SSP) to help breed and conserve the rare toad.	
Columbus	Puerto Rican crested toad ( <i>Peltophryne lemur</i> ). Loss of coastal wetlands due to development, conversion to crop land and drainage for mosquito control, threaten this amphibian with extinction. Survival depends upon habitat protection and captive breeding and reintroduction plans. The Columbus Zoo participates in a Species Survival Plan for the Puerto Rico and Virgin Gorda. Habitat: exposed limestone outcroppings.	They like to squeeze between the rocks, but they can't go back very far. Rocks are stacked to leave cracks that the toads utilise, but are still visible. There is also a glass petri dish that is buried to its rim and filled with sheet moss that the toads sit in if the moss in not too wet.
Detroit	Once thought to be extinct in the wild, this small toad with bony ridges above its eyes is known to have a very small population on the island of	Put water up front; they like to sit in a water bowl.

**Appendix C (cont.)                      Part 1: Graphics and Exhibit Technique**

INSTITUTION	GRAPHICS	SECRET TO EXHIBITING TOADS
Detroit (cont.)	Puerto Rico. Captive-bred offspring of this endangered amphibian have been taken back to Puerto Rico for release into their native habitat. Tiny radio transmitters "backpacks" have been placed on some individuals by researchers to track the movements of these animals in the limestone crevices where they reside. Puerto Rican crested toad ( <i>Peltophryne lemur</i> ). Range: Puerto Rico. Also SSP logo on graphic. The exhibit in the amphibian center will have a continuously running loop playing above the exhibit showing habitat, backpacked animals, as well as traditional graphics.	
Fort Worth	Puerto Rican crested toad ( <i>Peltophryne lemur</i> ) Limestone karst formations, Puerto Rico and Virgin Gorda. Once believed to be extinct, there have been recorded sightings of this rare toad in the wild. SPECIES SURVIVAL PLAN. Zoos and aquariums throughout the world are currently working together through the AZA to protect and conserve the world's rare and endangered species. Through captive breeding and education, the SSP was established by the AZA to ensure viable gene pools of selected endangered species. The dream of all dedicated zoo and aquarium professionals is to see a time when, through captive propagation, stable populations of animals may be reintroduced to protected natural habitats. The members in this exhibit are members of the Species Survival Plan.	Shallow limestone "caves" are provided. Longer more narrow caves allowed toads to hide too much. When filled in with gravel, the toads started scraping there snouts. Caves were then enlarged and their snouts healed up and the public can still see them.
Louisville	The Puerto Rican Crested Toad was the first amphibian to become part of the American Zoo and Aquarium Associations Species Survival Plan. For more than 30 years it was thought to be extinct. The larger Cane Toad was introduced in the early 1900's and competes with the Puerto Rican crested toad for resources. Due to human development, toad populations have disappeared in many areas. The largest breeding site for this species is a dirt parking lot under constant threat of development. Also a range map, diet info and a toad sticker.	Opening up crevices in front and closing them off in the back. Also place pool in front of exhibit.

**Appendix C (cont.)                      Part 1: Graphics and Exhibit Technique**

<b>INSTITUTION</b>	<b>GRAPHICS</b>	<b>SECRET TO EXHIBITING TOADS</b>
Philadelphia	It is hard to believe that up until 1967 everyone thought the Puerto Rican crested toad was extinct. Today we released some more tadpoles that were born at the Toronto Zoo. Starting with six toads, zoos have returned 4,000 captive-bred toadlets and 12,000 tadpoles to the wild. The Philadelphia Zoo is building artificial breeding ponds in Puerto Rico to continue this success.	Rocks are stacked securely to provide dimly lit low caves. Caves must be low enough so the toads have to hunker down to fit. Tank is constantly moist using a magnum 350 filter for recirculation of water.
St. Louis	Focus on conservation. The crested toad is only found in Puerto Rico. This species is endangered of extinction and depends on the limestone habitat along the north and south-western coasts of the island. Heavy seasonal rains trigger the toads to emerge from the limestone and breed in the temporary ponds formed by the rain. There is very little freshwater habitat remaining in these areas and the toads now breed in cement cattle troughs that are filled by rainwater. Freshwater ponds are essential for the survival of this species. Imagine waiting all year to breed, only to discover that the pond is dry!	The rockwork in the exhibit is set up in such a way that the animals feel secure, but are visible by the public.
Santa Barbara	Listed as a threatened species, the Puerto Rican crested toad is part of the SSP. This program attempts to maintain the diversity of each species listed through a strict breeding program. Range...	Have tried placing curved bark against glass or positioned so that the only cover was under it and the animals could be viewed. After a day or so they found a way of moving the bark or positioning themselves out of sight. Have also used minimal substrate.
Sedgwick County	SSP - Those initials stand for Species Survival Plan. SSP is a way for co-operating zoos to manage the captive breeding of endangered species on an international scale. The object is to avoid inbreeding and to weed out harmful inherited traits. The Puerto Rican crested toad is one of this zoo's SSP species.	Rock crevices large enough to squeeze into, but shallow enough so the toad can't completely conceal itself -- a difficult balance to achieve; a "rain" period will bring them out of hiding for a while.

## Appendix C (cont.)      Part 1: Graphics and Exhibit Technique

INSTITUTION	GRAPHICS	SECRET TO EXHIBITING TOADS
Toronto	Conservation - how precarious survival is; role of community in conservation and role of zoos (SSP) in assisting survival (release to adjacent constructed ponds).	Will provide limited rock depressions shallow enough to hide in but remain visible. Plastic tubes will be designed to keep toads in clear part of the tube, but secure in the "tunnel".
Vancouver Aquarium	You are looking at some very lucky toads. They were thought to be extinct until 1967. Fortunately there were enough of them left to start a captive-breeding program in 1982. Our Aquarium is proud to be one of the zoos and aquariums breeding these toads under a Species Survival Plan. So far, 16,000 captive-bred tadpoles and toadlets have been released back to Puerto Rico. These toads are now listed as threatened by the U.S. Fish and Wildlife Service – Not bad for toads thought to be extinct just over 30 is the loss of their breeding ponds and limestone-hill homes to residential and tourist developments.	The sandstone slabs are stacked so most of the hiding spots are somewhat visible to the public. Most of the time the toads' heads are visible, and the visitors get a pretty good view.

## Part 2: Exhibit Detail

Institution	Dimensions	Substrate	Plants	Props	# of toads	Other Information
Buffalo	4' wide 3' high 2' deep	Topsoil mixed with water and allowed to dry.	Pear cacti and other species of cacti	Cacti, hawthorn branches (dead), stone bunkers pushed into mud when soft	3.2 - 4.5	Tried several other mixes of substrate; if it was loose at all, the toads disappeared. The mud worked great in my opinion; you can even imbed rocks in the mud and make it look like a limestone hillside. Cacti pots were buried in the mud as well.
Calgary	40" wide 65" high 38" deep	Rock, moss, gunnite	Asparagus fern, pothos, silver king	moss, slate	2.4	



**Appendix C (cont.)**

**Part 2: Exhibit Detail**

<b>Institution</b>	<b>Dimensions</b>	<b>Substrate</b>	<b>Plants</b>	<b>Props</b>	<b># of toads</b>	<b>Other Information</b>
Cleveland Metroparks	3' wide 5' high 3' deep	Fibre glass	Phiodendron, bromeliad	2" - 3" washed river rocks, native wood branches, and sheet moss.	3.0.0	
Columbus	13" wide 16" high 36" deep	Dry topsoil	Tillandia species (air plants - bromeliads) placed in rock crevices.	Back and side walls are covered by tuffa rock stacked to the top. Crevices are filled with sheet moss.	0.1 - 0.3	
Detroit	12" wide 10" high 14" deep	Sharp sand	Succulents	Limestone rock	2.0.0	
Fort Worth	38" wide 19" high 19" deep	Gravel (white limestone gravel mixed with brown and tan gravel)	Asparagus fern	Limestone rocks, sand-stone, twig-like branches and a slow waterfall	1.1	
Louisville	24" wide 14" high 18" deep	Soil, gravel, rock, moss, bark	Creeping asparagus	Limestone, sheet moss cork bark.	0.3	
Philadelphia	24" wide 14" high 36" deep	Pea gravel	Pothos	Limestone rock	3.0.0	
St. Louis	87.6 cm 45.7 cm 55.8 cm	Gravel mixture, soil in areas where there are plants	Various types of ivy, pothos, and ficus. Future exhibit will have arid adapted plants.	Rocks, driftwood.	0.3	
Santa Barbara	11" wide 12" high 12" deep	Bark, moss	Cut plants from zoo grounds that are changed every 1-2 days.	Bark and rocks.	2.0.0	

**Appendix C (cont.)**

**Part 2: Exhibit Detail**

<b>Institution</b>	<b>Dimensions</b>	<b>Substrate</b>	<b>Plants</b>	<b>Props</b>	<b># of toads</b>	<b>Other Information</b>
Sedgwick County	23" wide 24" high 19" deep	Medium size gravel in pool area, lime-stone rocks cover terrestrial portion.	Sanseveria, hoyo, pothos, Swedish ivy.	Twigs, dried grass	4.0.0	Exhibit has a floor drain to allow pool water changes (about 3 X a week). Animals are rotated on and off exhibit.
Toronto	18" wide 2' high 3' deep	Shallow bark mulch and 1/2" river stone for drainage.	1 plastic cactus	Limestone rock, clear plastic tubes as tunnels, cactus wood.	3.3	
Vancouver Aquarium	40 cm wide 40 cm high 80 cm deep	Soil covered with sandstone slabs (toads cannot get to soil).	Pothos, spathiphyllum ficus, pumilla	Rocks and plants	1.1	Although the sandstone is visually nothing like the toads natural habitat, I feel it allows me to reproduce the environmental conditions fairly accurately, and is easily cleaned and maintained.

Appendix D

INSTRUCTIONS FOR DOMESTIC SHIPMENTS

**WITHIN THE UNITED STATES**

Shipping will be relatively simple. Book your shipment with the carrier of your choice, ensure the shipping container is of IATA standard, labels are affixed properly, & emergency instructions are affixed to the outside of the shipping container.

Other documents to include with the shipment are, specimen reports, emergency instructions, IATA declaration.

There are no permits required to move the toads interstate, between participating institutions as long as there is no commerce between exchanging institutions. USF&W retains ownership of the toads. As these toad shipments are for breeding and relocation/reintroduction, and involves no commerce, no permits are needed. It would be a good idea to include with your shipment a copy of the USF&W Puerto Rican Crested Toad permit, to confirm that the institutions involved in the shipment are on the list of approved institutions to hold and relocate toads.

**WITHIN CANADA**

Follow the information give above for the United States. Check with the local Provincial Wildlife or Natural Resources Ministry to confirm there are no import or export permits required by the Province. Some Provinces in Canada will require a permit from them to move the toads into or out of the Province.

## **Appendix E**

## **INSTRUCTIONS FOR INTERNATIONAL SHIPMENTS**

### **DOCUMENTS REQUIRED:**

- a valid copy of the USF&W Puerto Rican Crested Toad Permit (for import/export from or to the USA)
- health certificate
- Airway bill—available from the airline and usually filled out by airline staff
- USFW 3-177 form (import / export declaration )
- IATA declaration
- Commercial invoice also known as Proforma invoice
- Emergency instructions

### **FLIGHT BOOKING:**

The airline of choice is Delta Airlines. Call the Delta Air Cargo office and make you're booking 4-5 days in advance of the proposed shipping date. Ensure your shipment is to go "DELTA DASH" (this is a priority service). You will need to book to enter at one of the designated ports for wildlife to enter the USA. We recommend you use Atlanta, as it most suitable to favourable weather conditions at the time of year shipping occurs.

The shipment will be done over two days, day one the shipment arrives in Atlanta at or about noon or early afternoon to allow for USFW inspection. The shipment will then sit overnight and take the first Delta flight out of Atlanta for San Juan, Puerto Rico, arriving San Juan about noon. This allows USFW staff time to clear customs and pickup and deliver to the release sites before nightfall.

### **NOTIFICATION OF CONSIGNEE AND USFW ATLANTA**

#### **USFW Service:**

Once you have booked the shipment and received an airway bill number, proceed to complete all the required paperwork. Then notify by phone (follow up with fax) the USFW Service at Atlanta for the inspection. Send to them via fax a copy of the permit, airway bill, and a copy (unsigned) of the USFW 3-177 form, and any other information they may require.

US Fish & Wildlife Service, Atlanta Airport: Inspector Kenneth Endriss Ph: 404-763-7959  
Fax: 404-763-7560.

#### **CONSIGNEE:**

Ensure the address for the consignee is as follows:

US Fish & Wildlife Service Attn: Ken Foote (787) 851-7297 x 29

Carr.301, Km 5.1

P.O. Box 491

Boqueron, Puerto Rico, USA. 00622

AND: Miguel Canals, Dept. of Natural Resources, Guanica Forest, (787) 821-5706.

Send to Ken Foote all the information regarding the shipment. This will include the flight details, time of arrival, etc. Also send with the information a copy of the USFW 3-177 (unsigned copy) and ask Ken Foote to sign and forward to the inspectors at the Atlanta Airport. US law requires the consignee (in this case the USFW, Puerto Rico) to sign the 3-177 at the port of entry

## **Appendix E (cont.)            INSTRUCTIONS FOR INTERNATIONAL SHIPMENTS**

(Atlanta). Ken Foote will need to liase with the USFW inspectors in Atlanta to receive the signed copy. This signed copy is required for USFW clearance.

### **ATTACHED DOCUMENTS AND HOW TO COMPLETE:**

- **AIRWAYBILL:**

Box 1: (Routing and destination)

Note: the airlines use codes for airports ATLANTA is known as “ATL, SAN JUAN is known as “SJU” fill in the TO box with the letters “ATL”, the next box is “by first carrier” fill in Airline taking he shipment, in this case Delta Airlines. The next box is TO fill in the letters SJU for San Juan, the next box is labelled BY fill in the Airlines Code, Delta is DL, Drop down to the Airport of Destination and enter San Juan Puerto Rico,

Box 2:

This is where you will fill in the address of the USFW Boqueron, Puerto Rico with Ken Foote as the contact and her phone number

Box 3:

Fill in the shippers name and address along with a contact name and phone number and enter your account number with Delta in the box as indicated

Box 4:

Fill in the box only indicated “Airport of Departure” this is the name of the departure airport

Box 5:

Enter the currency of the shipment i.e.: for a value in US Dollars enter “USD, for a value in Canadian Dollars enter “CND”. If the shipment is to be sent prepaid, enter an XX in the box indicated by PPD. Enter the declared value for carriage and declared value for customs in the boxes as indicated. Note: this value must be the same in both cases.

Box 6:

Enter the number of pieces as indicated, move across the column as indicted Nature and quantity of goods and enter “LIVE TOADS/TADPOLES”; under this enter the dimensions of the boxes in the shipment.

Box 9:

Enter any handling information, see example

Sign airway bill at the bottom as indicted by “signature of shipper above” and initial the box indicating this shipment does not contain dangerous goods.

**Appendix E (cont.)      INSTRUCTIONS FOR INTERNATIONAL SHIPMENTS**

- US FISH & WILDLIFE SERVICE 3-177 --DECLARATION FOR IMPORTATION OF EXPORTATION OF FISH OR WILDLIFE

See attached copy for a sample of how to fill in

- COMMERICAL INVOCIE OR PROFORMA INVOICE

See attached copy of how to complete, Note: value on this form must be the same as entered on the airway bill in the boxes indicated “ declared value for carriage and declared value for customs”

- IATA SHIPPER’S CERTIFICATION FOR LIVE ANIMALS

See attached copy

- EMERGENCY INSTRUCTIONS

See attached copy of our emergency instructions; you should include this information with the paperwork and also attach a copy to outside of one of the boxes in the shipment.

**TO TRACK INTERNATIONAL SHIPMENTS:**

To track an international shipment, go to the Delta Air Cargo web site (<http://www.delta-air.com/home/index.jsp>) and find the area indicated “Site map and Search” and click on it. Your will be brought to a screen listing “Programs and Services” click on "Delta Air Cargo", a screen will appear where you can enter your airway bill to enable you find out the status of your shipment.



**Toronto Zoo**

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Scarborough, Ontario, Canada M1B 5K7  
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www.torontozoo.com

Councillor Raymond Cho - Chairman Calvin White - General Manager & CEO

## EMERGENCY INSTRUCTIONS - ANIMAL SHIPMENTS

### SPECIES: PUERTO RICAN CRESTED TOAD TADPOLES

**FOOD:** if delayed more than 12 hours feed animals with small amounts of food listed below:

- 1) see attached diet sheets or instructions below
- 2)
- 3)
- 4)
- 5)

**WATER:** available at all times

**DRUGS OR MEDICAL AID:**

TO BE ADMINISTERED BY A QUALIFIED ATTENDANT OR ANIMAL CARE EXPERT

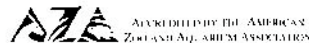
**EMERGENCY CONTACTS/PHONE NUMBERS:**

METRO TORONTO ZOO, VETERINARIAN, PH: 416-392-5901

ELDON SMITH, CURATORIAL ASSISTANT,  
ANIMAL IMPORTS/EXPORTS, METRO TORONTO ZOO,  
Monday to Friday, 8:30am to 4:00pm, 416-392-5997; 416-392-5901.  
(after hours 905-640-5850)

AFTER HOURS (6:00pm to 6:00am), METRO TORONTO ZOO, SECURITY, 416-392-5904

**OTHER:** KEEP IN COOL, QUIET AREA. ANIMALS EXTREMELY SENSITIVE





**Toronto Zoo**

361A Old Finch Avenue,  
Scarborough, Ontario, Canada M1B 5K7  
Tel. (416) 392-5900 Fax (416) 392-5934  
www.torontozoo.com

Councillor Raymond Cho - Chairman Calvin White - General Manager & CEO

**COMMERCIAL INVOICE**

**TO WHOM IT MAY CONCERN:**

Consignment of : 1000 Puerto Rican Crested toads tadpoles (*Peltophyryne lemur*)

to: US Fish & Wildlife Service, Boqueron PR. USA. 00622  
(complete street address)

is a return of tadpoles owned by USFW Service for reintroduction to the wild from the Toronto Zoo.

No monies are involved.

Value for customs purposes only: \$100.00

Province of Origin: XO (Ontario)

Consignee IRS/SSNumber:  
(Internal Revenue service/social security no, US only)

Approximate wt of shipment: 20 lbs (10 kgs)

AIRWAYBILL # 006-5328-9806

DATE 22 November 1999

I CERTIFY THAT THIS DECLARATION IS TRUE AND CORRECT

Eldon W. Smith  
Curatorial Assistant  
Imports/Exports  
Metro Toronto Zoo.



CAZAG  
Canadian Association  
of Zoos and Aquariums  
Address: 1000 University Ave.  
Toronto, Ontario M5G 1A5



AZA  
Associated with the American  
Zoological Association





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**SHIPPER'S CERTIFICATION FOR LIVE ANIMALS**

(to be completed in duplicate)

This is to certify that (check appropriate box):

- ☐ In addition to having completed all advance arrangements, this consignment is properly described and packed, and is in proper condition for carriage by air according to the current edition of the IATA Live Animals Regulations and all applicable carrier and governmental regulations. The animal(s) of this consignment is (are) in good health and condition.
- ☐ Animals taken from the wild for shipment have been appropriately acclimatised.
- ☐ This consignment does not include Appendix I species as described in the Convention on International Trade in Endangered Species of Wild Fauna and Flora or in other applicable national legislations. Applicable permits/certificates are attached to the air waybill.
- ☐ The endangered species contained in this consignment can be legally carried and imported into the country of ultimate destination and through the transit countries en route.

The shipper accepts that carriers will not be liable for any loss, damage or expense arising from death due to natural causes, or death or injury of any animal caused by the conduct or acts of the live animal itself or of other animals, such as biting, kicking, goring or smothering, nor for that caused or contributed to by the conditions, nature or propensities of the animals. In no event will carrier be liable for death or injury to an animal attendant caused or contributed to by the condition, conduct or acts of animals.

Number of Package(s)	Specific Container Requirement Number (see IATA Live Animals Regulations)	Species (description and names — scientific and common) and Quantity of Animals

Name and address of shipper ..... Signature of shipper ..... Date..... Year/Month/Day (See reverse side for special conditions)	Shippers failure to comply in all respects with the applicable IATA Live Animals Regulations and any other international and/or national government regulations, may be in breach of applicable law and subject to legal penalties.	
Air Waybill No.	Airport of Departure	Airport of Destination



**CAZA** Canadian Association of  
Zoo & Aquariums  
Association canadienne des  
parcs zoologiques & aquariums




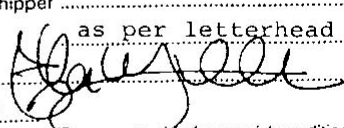
ACCREDITED BY THE AMERICAN  
ZOO AND AQUARIUM ASSOCIATION



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 <b>SHIPPER'S CERTIFICATION FOR LIVE ANIMALS</b> <i>(to be completed in duplicate)</i>		
<p>This is to certify that (check appropriate box):</p> <p><input checked="" type="checkbox"/> In addition to having completed all advance arrangements, this consignment is properly described and packed, and is in proper condition for carriage by air according to the current edition of the IATA Live Animals Regulations and all applicable carrier and governmental regulations. The animal(s) of this consignment is (are) in good health and condition.</p> <p><input type="checkbox"/> Animals taken from the wild for shipment have been appropriately acclimatised.</p> <p><input checked="" type="checkbox"/> This consignment <i>does not</i> include Appendix I species as described in the Convention on International Trade in Endangered Species of Wild Fauna and Flora or in other applicable national legislations. Applicable permits/certificates are attached to the air waybill.</p> <p><input checked="" type="checkbox"/> The endangered species contained in this consignment can be legally carried and imported into the country of ultimate destination and through the transit countries en route.</p> <p>The shipper accepts that carriers will not be liable for any loss, damage or expense arising from death due to natural causes, or death or injury of any animal caused by the conduct or acts of the live animal itself or of other animals, such as biting, kicking, goring or smothering, nor for that caused or contributed to by the conditions, nature or propensities of the animals. In no event will carrier be liable for death or injury to an animal attendant caused or contributed to by the condition, conduct or acts of animals.</p>		
Number of Package(s)	Specific Container Requirement Number (see IATA Live Animals Regulations)	Species (description and names — scientific and common) and Quantity of Animals
2	#51	1000 Puerto Rican Crested Toad tadpoles
		( <i>Peltophryne lemur</i> )
<p>Name and address of shipper ..... as per letterhead</p> <p>Signature of shipper ..... </p> <p>Date 1999/11/23 Year/Month/Day (See reverse side for special conditions)</p>		<p>Shippers failure to comply in all respects with the applicable IATA Live Animals Regulations and any other international and/or national government regulations, may be in breach of applicable law and subject to legal penalties.</p>
Air Waybill No. 006-5328-9806	Airport of Departure TORONTO - YYZ	Airport of Destination San Juan Puerto Rico SJU




**CAZA** Canadian Association of  
Zoos & Aquariums  
Association canadienne des  
parcs zoologiques & aquariums



ACCREDITED BY THE AMERICAN  
ZOO AND AQUARIUM ASSOCIATION

USFWS Form 3-117  
(revised 8/92)  
Form Approved O.M.B. No. 1018-0042  
Appraisal Expires 4/3/99

**U.S. FISH AND WILDLIFE SERVICE**



**DECLARATION FOR IMPORTATION  
OR EXPORTATION OF  
FISH OR WILDLIFE**

Name of Carrier:

Airway Bill or Bill of Lading No.:

Imported or Exported Via:

☐ air cargo ☐ ocean cargo ☐ truck  
☐ rail ☐ mail ☐ personal baggage  
☐ automobile: License no. \_\_\_\_\_  
state \_\_\_\_\_

Location where wildlife is available for inspection:

Package or Bale Marks and Nos.:

Indicate One

☐ Import ☐ Export

Port of:

Date:

☐ Commercial ☐ Non-commercial

Customs Identification No.:

Please Type or Print Legibly

(Indicate one)

☐ U.S. Importer of Record  
☐ U.S. Exporter \_\_\_\_\_  
(name) \_\_\_\_\_  
(address - street, city, state, zip code)

Foreign Consignor  
or Consignee \_\_\_\_\_  
(name) \_\_\_\_\_  
(address - street, city, country)

Customs Broker  
or Agent \_\_\_\_\_

Shipping Agent or  
Freight Forwarder \_\_\_\_\_

Furnish All Information Below (invoices or lists providing required information may be attached)

QUANTITY	SCIENTIFIC NAME Genus Species	COMMON NAME	DESCRIPTION If live, so state. If product, describe.	DOMESTIC VALUE	COUNTRY OF ORIGIN

U.S. License and/or Permit Nos.:

Foreign License and/or Permits:  
Country \_\_\_\_\_ No. \_\_\_\_\_

I certify the information contained herein is true and complete to the best of my knowledge and belief.

\_\_\_\_\_  
signature

\_\_\_\_\_  
date

Action Taken

☐ cleared  
☐ clearance refused  
☐ seized

\_\_\_\_\_% of Wildlife Inspected

Officer Signature and Badge No. \_\_\_\_\_

**FOR OFFICIAL USE ONLY**

Carrier Number

SEE REVERSE OF THIS FORM FOR INSTRUCTIONS AND PRIVACY ACT NOTICE

U.S. FISH AND WILDLIFE SERVICE



DECLARATION FOR IMPORTATION  
OR EXPORTATION OF  
FISH OR WILDLIFE

Indicate One:  
☒ import ☐ export  
Port of: Atlanta, GA  
Date: 1999/11/23  
☐ Commercial ☒ Non-commercial  
Customs Identification No: \_\_\_\_\_

Name of Carrier:  
Delta Airlines  
Airway Bill or Bill of Lading No.:  
006-5328-9806  
Imported or Exported Via:  
☒ air cargo ☐ ocean cargo ☐ truck  
☐ rail ☐ mail ☐ personal baggage  
☐ automobile: License no. \_\_\_\_\_  
state \_\_\_\_\_  
Location where wildlife is available for inspection:  
Atlanta GA  
Package or Bale Marks and Nos.: \_\_\_\_\_

(Indicate one)  
☒ U.S. Importer of Record  
☐ U.S. Exporter USFW Service, P.O.Box 491, Boqueron Puerto Rico, USA. 00622  
(name) (address - street, city, state, zip code)  
Foreign Consignor or Consignee Toronto Zoo, 361A-Old Finch Ave., Scarborough ON. M1B 5K7  
(name) (address - street, city, country)  
Customs Broker or Agent \_\_\_\_\_ Shipping Agent or Freight Forwarder \_\_\_\_\_

Furnish All Information Below (Invoices or lists providing required information may be attached)

QUANTITY	SCIENTIFIC NAME Genus Species	COMMON NAME	DESCRIPTION If live, so state. If product, describe.	DOMESTIC VALUE	COUNTRY OF ORIGIN
approx 1000	<u>Peltophryne lemur</u>	<u>Puerto Rican crested toad</u>	<u>LIVE TADPOLES</u>	<u>\$100.00</u>	<u>ch. Canada</u>
the above toad tadpoles were captive hatch at the Toronto Zoo, Nov '99, for reintroduction into the wild in Puerto Rico					

U.S. License and/or Permit Nos. PRT-842524  
SA-97-25  
Foreign License and/or Permits: NONE  
Country \_\_\_\_\_ No \_\_\_\_\_  
I certify the information contained herein is true and complete to the best of my knowledge and belief  
signature \_\_\_\_\_ date \_\_\_\_\_

Action Taken \_\_\_\_\_ Date \_\_\_\_\_  
☐ cleared  
☐ clearance refused  
☐ seized  
\_\_\_\_\_% of Wildlife Inspected  
Officer Signature and Badge No \_\_\_\_\_

FOR OFFICIAL USE ONLY

Control Number:

SEE REVERSE OF THIS FORM FOR INSTRUCTIONS AND PRIVACY ACT NOTICE



006- 5 328 9784		0412 90077 Rev. 3/92 AG-13 5P 850		006- 5 328 9784	
SHIPPER'S NAME AND ADDRESS Toronto Zoo 361A Old Finch Ave Scarborough ON, Canada M1B 5K7 Eldon Smith 416-392-5997		SHIPPER'S ACCOUNT NUMBER		NOT NEGOTIABLE <b>AIR WAYBILL</b> (AIR CONSIGNMENT NOTE) <b>DELTA</b> Copies 1, 2 and 3 of this Air Waybill are originals and have the same validity. It is agreed that the goods described herein are accepted in apparent good order and condition (except as noted) for carriage SUBJECT TO THE CONDITIONS OF CONTRACT ON THE REVERSE HEREOF. THE SHIPPER'S ATTENTION IS DRAWN OF THE NOTICE CONCERNING CARRIER'S LIMITATION OF LIABILITY. Shipper may increase such limitation of liability by declaring a higher value for carriage and paying a supplemental charge if required.	
CONSIGNEE'S NAME AND ADDRESS USFW Service Carr, 301, Km 5.1, P.O.Box 491 Boqueron, Puerto Rico, USA. 00622 Susan Silander 787-851-7297		CONSIGNEE'S ACCOUNT NUMBER		TO EXPEDITE MOVEMENT, SHIPMENT MAY BE DIVERTED TO MOTOR OR OTHER CARRIER AS PER TARIFF RULE UNLESS SHIPPER GIVES OTHER INSTRUCTIONS HEREON.	
ISSUING CARRIER'S AGENT NAME AND CITY f		ALSO NOTIFY NAME AND ADDRESS (OPTIONAL ACCOUNTING INFORMATION)			
AGENTS IATA CODE		ACCOUNT NO.		ACCOUNTING INFORMATION	
AIRPORT OF DEPARTURE (ADDR OF FIRST CARRIER) AND REQUESTED ROUTING Lester B. Pearson Intl - TORONTO -					
AIRLINE AND CARRIER By First Carrier ATL Delta Airlines		CND		DECLARED VALUE FOR CARRIAGE \$100.00 DECLARED VALUE FOR CUSTOMS \$100.00	
AIRPORT OF DESTINATION San Juan, P.Rico		FLIGHT DATE		AMOUNT OF INSURANCE (If shipper requests insurance in accordance with conditions on reverse thereof, declare amount to be insured in box marked "Amount of Insurance")	
HANDLING INFORMATION These commodities licensed by US for ultimate destination. Dimension contrary to US law is prohibited. USFW clearance will be done in ATL. Arrangements have been made, consignee has been notified of shipment.					
NO. OF PIECES RCP		GROSS WEIGHT		NATURE AND QUANTITY OF GOODS (INCL DIMENSIONS OR VOLUME)	
2				LIVE TOADS/ TADPOLES 2 @ 20" x 20" x 16"	
PREPAID		WEIGHT CHARGE		COLLECT	
A.		B.		C.	
VALUATION CHARGE		DELIVERY CHARGES		OTHER CHARGES AND DESCRIPTION	
D.		E.		F.	
TOTAL OTHER CHARGES DUE AGENT		Shipper certifies that the particulars on the face hereof are correct and that insofar as any part of the consignment contains restricted articles, such part is properly described by name and is in proper condition for carriage by air according to applicable national government regulations and for international shipments, the current International Air Transport Association's Dangerous Goods Regulations.			
TOTAL OTHER CHARGES DUE CARRIER		Toronto Zoo/ SIGNATURE OF SHIPPER ABOVE AND INITIAL APPLICABLE BOX BELOW <input type="checkbox"/> This shipment does not contain dangerous goods regulated in air transport <input type="checkbox"/> This shipment does contain dangerous goods regulated in air transport			
COD → CURRENCY		EXECUTED ON			
TOTAL PREPAID		TOTAL COLLECT		SIGNATURE OF ISSUING CARRIER OR ITS AGENT	
CURRENCY CONVERSION RATES		TOTAL COLLECT IN DESTINATION CURRENCY		(Date) (Time) at (Place)	
FOR CARRIERS USE ONLY AT DESTINATION		CHARGES AT DESTINATION		3-Original For Shipper	
All Collect Charges in Destination Currency		TOTAL COLLECT CHARGES		006- 5 328 9784	

Appendix F

REPORT ON ANALYSIS OF ALGAE AND CYANOBACTERIA IN SAMPLES FROM  
SITES IN PUERTO RICO RELATED TO THE PUERTO RICAN CRESTED TOADS

J. Acreman, Curator

University of Toronto Culture Collection of Algae and Cyanobacteria (UTCC)

Department of Ecology and Evolutionary Biology

University of Toronto, Toronto, ON M5S 3B2

Tel: (416) 978-3641

Fax: (416) 978-5878

jacreman@eeb.utoronto.ca

October 20, 2006

Analysis of Algae and Cyanobacteria

Part 1:

Sample # 1. About 200 ml of fresh sample was collected June 5, 2006 from a captive rearing enclosure for Puerto Rican toads at the Toronto Zoo. It was held at 20 C in dim light until analyzed by the curator at the University of Toronto Culture Collection of Algae and Cyanobacteria (UTCC).

METHOD:

Two ml of the fresh sample were examined live under a Wild-Leitz inverted microscope using magnifications of 64X to 625X with brightfield and phase contrast. Slide mounts were also prepared and examined under 1000X (oil immersion) phase contrast. Most of the material in the original sample was a dense dark blue-green mat which consisted of several species of predominantly filamentous cyanobacteria. Rough estimates were made of the proportions of each taxon according to their contribution to the biomass. The following taxa were found:

BACILLARIOPHYCEAE

Nitzschia sp. (few <1%)

CYANOPHYCEAE

Anabaena sp.1 (20 %)

Anabaena sp. 2 (5%)

Gleocapsa sp. (2%)

Leptolyngbya cf. boryana sp. (dominant: 60 %)\*

Leptolyngbya sp. (2%)

Scytonema sp. (10 %)

#### CHRYSOPHYCEAE

Mallomonas sp. (few ~1 %)

In addition there were numerous zooflagellates and non-photosynthetic bacteria.

\* This species will be isolated into pure culture at the UTCC for further examination. Time permitting, other species from this sample will also be isolated into pure culture.

#### Part Two:

Five samples of algal mats were collected in the period November 15-22, 2005 from Puerto Rico but were frozen prior to delivery to the UTCC at University of Toronto. They were then held at 4C in the dark until examined. All of the samples contained mats with high amounts of sediment and very little moisture. The cells were badly degraded making the analysis difficult.

#### METHOD:

Samples of about 1 cm<sup>2</sup> were spread on slides and distilled water added to disperse the material. The entire slide was examined at magnifications of 64X to 625 X using phase contrast. Rough estimates were made of the proportions of each taxon according to their contribution to the biomass.

#### Sample # PR-17: Finca El Tallonal

The sample was taken from pond 1C in the sun. It consisted of mostly decayed vegetation which appeared to be from leaves. There were few algal cells present. A pale, filamentous cyanobacteria, probably *Leptolyngbya*, was present in high numbers but badly degraded. The following taxa were observed:

#### CHLOROPHYCEAE

*Kirchneriella* sp. (dominant 70%)

*Scenedesmus* sp. (5%)

#### CYANOPHYCEAE

*Leptolyngbya* sp.? (~ 25 % see above)

#### BACILLARIOPHYCEAE

*Navicula* sp. (few <1 %)

#### Sample # PR-18: Tamarindo

The sample was collected from a pond and consisted of mostly fine-grained sediment and few cells of algae and cyanobacteria. The following taxa were observed:

**BACILLARIOPHYCEAE**

Navicula sp. (25%)

Nitzschia sp. (25%)

**CHLOROPHYCEAE**

Geminella sp? (10%)

Scenedesmus sp. (25%)

Unidentified colonial green alga (5%)

**CYANOPHYCEAE**

Gleocapsa sp. (10%)

**Sample # PR-20 Tamarindo**

The sample was collected from the edge of a pond with marine toadlets. It consisted of fine grained sediment and the following taxa:

**BACILLARIOPHYCEAE**

Nitzschia sp. (25%)

**CHLOROPHYCEAE**

Scenedesmus sp. (40 %)

**CYANOPHYCEAE**

Gleocapsa sp. (15%)

Lyngbya sp. (20%)

**Sample # PR-24: Tamarindo**

This sample was collected from the center of the pond beside crested toadlets. It contained the following taxa:

**BACILLARIOPHYCEAE**

Navicula sp. (few < 1%)

**CHLOROPHYCEAE**

Geminella sp? (5%)

Pithophora sp. (large filamentous mat-forming alga: ~30% of biomass)

Scenedesmus sp. (30%)

**CYANOPHYCEAE**

Chroococcus sp.(10%)

Gleocapsa sp. (5%)

Lyngbya sp. (20%)



Sample # PR-30 Coamo

This sample was collected by a river where the toadlets were last seen. It consisted of fine-grained sediment and a more diverse set of algal taxa (diatoms and green algae), than in the other 4 samples. In contrast to the other 4 samples there were no Cyanophyceae observed. Taxa found:

**BACILLARIOPHYCEAE**

Cymbella sp. (10%)

Navicula sp 1. (dominant 40 %)

Navicula sp 2 (20%)

Pinnularia sp. (10%)

**CHLOROPHYCEAE**

Euastrum sp. (<1% )

Mougeotia sp. (10%)

Scenedesmus sp. (10%)

Glossary:

Cyanophyceae have chlorophyll a and carry out oxygen-evolving photosynthesis, they have traditionally been aligned with algae and, with regard for their characteristic color, called blue-green algae.

Chrysophyceae are small flagellates that are a yellowish brown color. Like diatoms it can form silica, but it is stored in a cyst within the cell instead of the cell wall. Chrysophyceae are somewhat photosynthetic, but it can also digest its own food.

Chlorophyceae are a large and important group of freshwater green algae.

Bacillariophyceae (diatoms) are a major group of eukaryotic algae, and are one of the most common types of phytoplankton. Most diatoms are unicellular, although some form chains or simple colonies. A characteristic feature of diatom cells is that they are encased within a unique cell wall made of silica.