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Status of the Puerto Rican crested toad

Peltophryne lemur

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The Puerto Rican crested toad *Peltophryne lemur* is the only amphibian to date to be included in the AAZPA Species Survival Plan (SSP). The endemic toad species of Puerto Rico, it was thought to be extirpated as recently as 1980 (Rivero *et al.*, 1980). Despite intensive herpetological canvassing of Puerto Rico, fewer than 50 specimens had been recorded in the literature at that time.

In 1980 Rivero collected 2.2 wild adults which subsequently spawned at the Jardin Zoologico de Puerto Rico. Progeny from the 1981 breeding were placed at the Buffalo Zoological Gardens and the Chicago Zoological Park (Brookfield Zoo).

In March 1982 the Jardin Zoologico de Puerto Rico hosted the Puerto Rican Crested Toad Symposium to discuss in detail the natural history, habits, captive management and future of the species. A field expedition to Quebradillas, mounted at the close of the symposium, collected a further 5.2.1 toads. A successful spawning at Puerto Rico Zoo resulted in toadlets being placed at the Buffalo and Indianapolis Zoos.

The AAZPA Wildlife Conservation Management Committee granted SSP designation to the Puerto Rican crested toad in 1984.

HISTORICAL BACKGROUND

The Puerto Rican crested toad has an interesting biogeographic and taxonomic

history. The species was first described as *Peltaphryne lemur* by Cope (1868) who reported on specimens collected for the Smithsonian Institution, Washington, DC. The generic name *Peltophryne*, which had been assigned (misspelt) by Cope, had been proposed earlier by Fitzinger (1843) for the casque-headed toads of the Greater Antilles. The specific name *lemur*, a reference to the nocturnal mammalian prosimian, reflected the nocturnal habits of the toad (Stejneger, 1904). Peters (1876), after examination of additional specimens, concluded that the Puerto Rican specimens were identical to the casque-headed *P. gutturosis* of Hispaniola.

Later Stejneger (1904) concluded that *lemur* was insufficiently distinct from *Bufo* to warrant inclusion as a separate genus and *Peltophryne* was relegated to a synonym of *Bufo*. He disagreed, however, with the conclusions regarding its standing as a population of *gutturosis* and reinstated its specific status as a Puerto Rican endemic.

While visiting Virgin Gorda in the British Virgin Islands, Barbour (1917) collected and described *Bufo turpis* but its similarity to *P. lemur* was not recognised. Schmidt (1928) extended the range of *P. lemur* in Puerto Rico and Grant (1932) made casual reference to an additional seven specimens of *turpis* taken by Danforth from Virgin Gorda in 1931. For over 50 years little else was published on *P. lemur* which was still considered a species of *Bufo*. Schwartz & Thomas (1975) relegated *turpis* to a synonym of *lemur*.

When Pregill's (1981) phylogenetic research, based on cranial osteological differences of the toads of the Greater Antilles, was completed the generic name *Peltophryne* was resurrected for *lemur*. Under the International Rules for Zoological Nomenclature, which dictate that the earliest available name proposed for a group be utilised in any taxonomic revision, *Bufo lemur* became known as *Peltophryne lemur*.

WILD STATUS

The crested toad is widely distributed in Puerto Rico (Schmidt, 1928) and specimens from both the Atlantic and Caribbean sides of the island have been recorded. Based on late Pleistocene fossil remains the toad's range is given as 'island wide at low elevations' (Pregill, 1981). Characterised as inhabiting semi-arid rocky areas of the island, the toad is closely associated with limestone Karst formations in well-drained soil (Schmidt, 1928; Rivero *et al.*, 1980).

Fossilised remains of the species are abundant and it has been speculated that its present rarity could be accounted for by its semifossorial existence coupled with inappropriate collection techniques (Pregill, 1981). Puerto Rico is the most populated of all the Antillean Islands; consequently, human encroachment and commercial development continue to pose a threat to this and other endemic species. Drainage and filling-in of breeding sites, and other disturbances of the habitat which led to loss of adults, prompted the US Department of the Interior Fish and Wildlife Service to designate the species as threatened in 1987.

The Department of Natural Resources, Puerto Rico, has requested that 50% of all captive-bred toadlets be returned to the island for release at sites selected to fall within the historic range but which do not overlap any existing populations. To date more than 2000 toadlets have been released. Because genetic over-representation and disease are major concerns, the survivability and eventual reproduction of reintroduced toadlets must be monitored. The optimum age at which the toads should be released to assure the greatest success needs yet to be investigated.

CAPTIVE MANAGEMENT

Despite its threatened status *P. lemur* is an easily maintained species; a protocol has been described by Paine (1985). The successful breeding programme developed by Metropolitan Toronto Zoo is the result of an approach to amphibian breeding

which regards the process as a sequence of elevating thresholds. As each threshold is reached, the animal is sensitised to the next. Thus an increase in moisture has an effect because of the dry period previously experienced.

The Toronto breeding programme begins a year in advance of the reproductive target date. Puerto Rican climate data and photoperiods for 20°N provide overall guidelines for the holding environment. The conditioning process prior to breeding requires about 60 days and breeding occurs five days after rehydration.

In December the quantity of food offered is increased and ♀♀ in particular receive newborn mice dusted with a calcium and vitamin D_3 supplement. Providing excess calcium prevents a drain on this mineral during vitellogenesis. At the end of January the aquarium is emptied and filled to a depth of 18 cm with a mixture of one-third peat moss and two-thirds sphagnum moss soaked with water until a friable mixture is formed. A variety of substrates have been used but in simultaneous trials the peat/sphagnum mix provided the largest margin of safety.

The top of the tank is screened to allow the gradual drying of the substrate (about 30 days at Toronto). A shallow water dish is available and its use can be monitored by the appearance of soil particles in it. As the substrate dries, if all is well with the toads, they should emerge in the evening to use the water which should therefore be dirty each morning.

During the 30-day drying process the toads are dug out and fed once a week in a separate tank to avoid ingestion of the substrate. Feeding ceases, usually voluntarily, about the time the substrate dries out to the bottom of the tank. The slow drying is facilitated by a relatively cool temperature (22°C) and moderate humidity (50–70%).

Depending on local temperature and humidity, each institution will have some variation in the rate and degree of drying. Obviously an awareness of how any bufonid behaves and appears during hibernation/aestivation assists in the intuitive judgement of the toads' health during the conditioning phase.

The toads are maintained under these 'dry' conditions for a further 30 days, during which disturbance should be kept to a minimum; the health of the animals can be monitored by dusting off the head and back to see that the toad is hydrated and not weak.

After 60 days the toads are slowly rehydrated by the addition of several litres of water twice daily for two days, and at the end of the second day the tank is sprayed until the peat is saturated. The air and water temperature is elevated to about 27°C.

On the morning of the third day ♂♂ are transferred to the breeding tank, which has been filled with water and an algal culture established. The tank is tilted so that there is 5–7·5 cm of water at one end and 15–17·5 cm at the other. In the deep end several submerged rocks, flower pots and plastic aquarium plants provide perching and egg-laying sites. A spray of water along one side of the tank provides a simulated rainfall. Tape-recorded calls are played to both sexes.

After the ♂♂ have been in the breeding tank for two days, the ♀♀ are introduced and amplexus will occur within a few hours if not immediately. The calls and rain are continued for three or four days. The Toronto staff have found that if there are no eggs within four or five days none will be laid.

Optimum mating and egg-laying occur if the toads are kept in a group until the eggs are laid since both sexes are stimulated by the wrestling of competing ♂♂. To confirm the identities of breeding pairs, however, and to prevent cross-fertilisation, the amplexing pairs are removed and housed separately. Once eggs are laid the adults are removed and the eggs are left to hatch *in situ*.

The best results are obtained with toads which are about two years old, the age when ♀♀ have assumed the necessary large

body mass and lipid reserves for reproduction. Attempts to breed three pairs which had not aestivated resulted in amplexus but no eggs were laid.

MARKING SYSTEM

Anuran species are notoriously difficult to mark permanently for identification purposes. Because of the permeability of amphibian skin, dyes or paints cannot be used and marking techniques have usually involved the amputation of toes used in conjunction with a numbering system. Tattooing, heat branding, silver nitrate branding or a variation of these have also been used. Although tattooing or heat branding may be permanent, the degree of stress to the animal during the application process can be severe and may have a negative effect on survival rates. Toe clipping is still the most widely used technique although it is not without drawbacks. Among these is the fact that it may be effective only in the short term since amputated digits are subject to regeneration (the degree of which is dependent on both the species and the stage of development when marked). In addition, the removal of one or more complete digits may adversely affect feeding and reproductive behaviours.

During 1986 the Buffalo Zoo began investigating alternative methods for marking anurans. Criteria previously published by Ferner (1979) were used to evaluate possible techniques. Some of the more applicable criteria were: the technique should not affect survivorship or behaviour; it should be as free from stress and pain as possible; marks should be easily identifiable and last the animal's lifetime; the technique should be relatively simple to use in both field and laboratory with costs kept to a minimum. With these criteria in mind, investigations were made into the use of freeze branding. The technique subsequently developed at the Buffalo Zoo was a variation on that used by Daugherty (1976) on *Ascaphus truei*. Freeze branding was also selected because of its possible application in future field studies on *Peltophryne lemur*.

Materials were similar to those used by Daugherty although the branding irons were different. Fabricated from 15-gauge copper, each iron was 140 mm long. Appropriate numbers were cut from 1·5 mm thick copper plating using a bandsaw. These were smoothed and shaped using fine sandpaper, and attached to the wire shafts by soldering.

Prior to the branding the irons were immersed in crushed dry ice for 30 minutes and subsequently reimmersed for one minute after each application. During branding the irons were removed from the dry ice and immediately applied to the skin surface, remaining there for approximately 30 seconds. Other than a slight reddening of the skin there were no marks immediately visible. However, after 24 hours the numbers were quite easily identified.

In some animals so marked, the numbers have remained for over two years. The best results were experienced with smooth-skinned anuran species such as *Bombina orientalis*. For species with a granular ventral skin surface such as *P. lemur* it is less effective and further experimentation with this technique is still necessary.

STUDBOOK

The studbook for *Peltophryne lemur* is maintained at the Buffalo Zoo by Frederick L. Paine, Curator of Birds and Reptiles. It contains the statistical history of the captive population (over 250 individuals) in 11 North American institutions since 1984. Information on releases to the wild are also included.

Entries are updated at least yearly. Animals reproduced in captivity are recorded initially as a spawn (to avoid assigning numbers to the hundreds of tadpoles) and surviving individuals are listed when they reach one year of age. At present, 45.38.34 live adults are registered.

DISEASES

Knowledge of the diseases of amphibians in general is still limited and this is particularly true for the Puerto Rican crested toad. There is as yet little documented evidence of any specific disease processes, although a survey into medical problems and pathology has been sent to participating institutions for future analysis.

The provision of a balanced diet is a constant problem in feeding small amphibians and the reliance on one type of food can lead to long-term deficiencies. Pathological fractures of the legs and spinal deformities (scoliosis) have been seen as suggestive of inadequate calcium (and possibly vitamin D) supplementation. Studies are under way at Toronto to determine if two syndromes, skeletal muscle degeneration and renal disease, have a nutritional basis.

As in other amphibians most bacterial infections would be expected to be caused by gram-negative organisms. Septicaemia and pneumonia have been recorded. Various protozoan parasites have been seen in captive specimens but their significance is unknown.

Mortality has occurred in several groups as a result of manipulation of animals for breeding purposes. Generalised desiccation can occur during pre-breeding aestivation. Conversely, when aestivated animals are rehydrated, excessive absorption of water into toads still experiencing renal shut-down can result in over-hydration and death.

GENETICS

Based on electrophoretic analysis of 28 liver proteins, captive Puerto Rican crested toad populations show a moderate to high genetic variability. Even though only a small number of clutches were examined, the populations do not show the lack of genetic variation that is typical of partially inbred populations. The genetic divergence observed between the southern samples and the northern samples is typical of geographically isolated populations of subspecies and may indicate geographic separation for a considerable length of time (up to one million years) but is not enough to indicate separate species.

SUMMARY

A general lack of knowledge of anuran husbandry has been emphasised through work with the Puerto Rican crested toad. Despite the intensive management programme that has been developed there are still many questions left unanswered about this as well as other anuran species. Relative newcomers to the world of captive management in zoological parks and aquariums, anurans are strikingly complex and standards of management must be developed to meet these complexities.

Future endeavours will aim at: (1) genetic management as determined by results of electrophoretic analysis of a larger sample size; (2) examination of the problem of calcium/phosphorus imbalance in the rearing of toadlets; (3) a new technique using microchips and electronic scanning to overcome the failings of the current marking systems; (4) a Master Plan for the conservation of the species.

In February 1989 expanded participation by institutions world-wide will be sought to diversify the captive population.

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A system for rearing tadpoles at the Buffalo Zoological Gardens

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Within the past decade the zoo community has shown an increasing interest in developing husbandry and programmes for anurans (Maruska, 1986). In the past this order of the Amphibia has been neglected primarily because of the specific and often difficult environmental conditions necessary for successful maintenance. With a greater understanding of these requirements, however, many more species are now being held and reproduced in zoos.

With increase in productivity comes the need for a time-efficient method of rearing the often staggering numbers of offspring that some members of this group are capable of producing. Using the method described in this paper, in 1987 the Buffalo Zoo reared through to metamorphosis 1707 tapoles from three anuran species, the Fire-bellied toad *Bombina orientalis,* White's tree frog *Litoria caerulea* and Puerto Rican crested toad *Peltophryne lemur.*

The rearing facility consists of 20 57 litre glass aquaria, measuring 60 × 31 × 32 cm high. Ten of the tanks were used for housing the tadpoles with the remaining ten used as reserve tanks for water changes.

Each holding tank is fitted with an open-top box insert, measuring 57·5 × 26 × 13 cm high. The sides of the boxes are constructed from 5 mm-thick plexiglas while the bottom is made from a fibreglass window screening, with a 1 × 1 mm

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