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Radiographic diagnosis of metabolic bone disease in captive bred mountain chicken frogs (Leptodactylus fallax)

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Complete List of Authors:	King, Jay; Rare Species Conservatory Foundation Muhlbauer, Michael; Veterinary Imaging Specialist, P.C. James, Arlington; Ministry of Agriculture and the Environment, Forestry, Wildlife & Parks Division
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INTRODUCTION

Leptodactylus fallax is a large terrestrial frog endemic to the island nations of the Commonwealth of Dominica and Montserrat. Commonly known as the mountain chicken frog, this species historically inhabited 8 islands in the Caribbean but now is considered critically endangered due to over hunting, habitat destruction, and chytridiomycosis in both countries, as well as volcanic activity on Montserrat [King, 2009]. As a result, captive breeding has become a high priority. Like most other amphibians, the nutritional requirements of this species during its different life stages are not known. As a result, captive specimens may suffer from metabolic bone disease (MBD).

The purpose of this study is to determine whether the nutritional needs of captive bred *L. fallax* had been met during its juvenile stages.

METHODS

Twelve wild adult *L. fallax* were captured in Dominica in 2001 and brought into captivity as part of a captive breeding program in conjunction with the Forestry, Wildlife and Parks Division of the Commonwealth of Dominica. Frogs were fed domestic crickets (*Acheta domesticus*) every other day. Crickets were fed a commercial cricket diet (Cricket Power Food ® Timberline Live Pet Foods) (Ca 4.18%, P 0.79%, Ca:P 5.3:1) [Dierenfeld and King, 2008] and dusted with a vitamin supplement (Rep-Cal ® Rep-Cal Research Labs) (Ca 35 - 45%, P 0%) prior to being fed to frogs. Decholorinated water was available at all times and changed at each feeding.

In 2005, two clutches of eggs were laid by two unrelated pairs of adult *L. fallax.* Offspring completed metamorphosis at approximately 6 ½ weeks of age. Froglets were fed crickets ad libitum from time of emergence from the nest until two years of age, at which time an every other day feeding schedule was initiated. Calcium glubionate (Calcionate Syrup ® Rugby) was added to the water at each water change.

Whole body radiography was performed on all frogs. Twenty-two captive bred subadult frogs (radiographed at 2 ½ years of age) and eleven adult wild caught frogs are included in this study. A dorsoventral view of each frog was submitted to a board certified veterinary radiologist. A comparison was made of skeletal structures between the two groups to assess bone density and abnormalities to aid in determining whether the nutritional needs of each frog were met during juvenile development. All frogs were without clinical signs of disease at the time of the study.

RESULTS

None of the wild caught frogs had radiographic evidence consistent with MBD. One frog had a healed fracture of the femur consistent with previous trauma. Wild caught frogs had normal appearing bone density and cortical thickness (Figure 1).

All of the captive bred frogs had radiographic evidence consistent with MBD (Figure 2). Multiple folding fractures of the long bones were present, averaging greater than 4 fractures per frog (Table 1). Fractures involved the femur, tibofibula and humerus. No fractures were observed in the phalanges, radioulna, or vertebrae. In addition to the fractures, all captive bred frogs had decreased bone density and thin boney cortices. The difference in bone density between the two groups was very evident in the skull. In wild caught frogs the large ocular orbits are well defined (Figure 3). Whereas, in captive bred frogs the bones surrounding the orbits were ill-defined and had irregular margins, appearing deformed (Figure 4).

DISCUSSION

Inadequate calcium is the most common nutritional deficiency in amphibians [Wright and Whitaker, 2001]**.** Prolonged hypocalcaemia often results in MBD with poorly mineralized or deformed bones (scoliosis, folding or pathological fractures), tetany, and rear limb paralysis [Green, 2001; Wright and Whitaker, 2001]. Common causes of MBD include diets deficient in calcium and diets with an inverse calcium to phosphorus ratio [Sabatini et al., 1998]. Vitamin D and ultra-violet light also play a major role in bone development by aiding in the absorption and retention of calcium. Deficiencies in these factors can result in MBD. Radiography is often valuable in assessing bone density and the presence of MBD.

Two methods are used in captivity to attempt to correct potential nutritional deficiencies: gut loading (food items, such as crickets, are fed calcium fortified diets) and dusting (coating foods items with powdered calcium supplements) [Sabatini et al., 1998]. Both of these methods are not without problems [Anderson, 2000]. While gut loading can increase the calcium level and calcium:phosphorus ratio, phosphorus levels are not affected. Dusting can increase the calcium and phosphorus levels, but supplements do not adhere for long periods and therefore, they must be consumed relatively quickly.

In this study, all captive bred frogs had radiographic evidence of MBD. Each frog had at least two fractures, with some having as many as six (Table 1). Most fractures involved the bones of the hind limbs: 95.5% of all tibio-fibulas and 97.7% of all femurs showed signs of old healed fractures. Fractures of the humerus were less common (22.7%). No fractures were seen in the radio-ulna. As suspected in this species, the locations of the fractures suggest that the bones of the hind limbs are under greater stress than those of the forelimbs during daily activities.

None of the wild caught frogs had radiographic evidence of MBD. This suggests that during development they obtained adequate nutrition (i.e., calcium, phosphorus, etc.) for normal bone health from their environment.

Folding fractures, as observed in the captive bred frogs in this study, are not traumatic fractures, but are the result of normal forces applied to each end of a soft bone resulting in a fold or bulge in the bone. This type of fracture often occurs in animals with nutritional deficiencies that interfere with normal bone calcification. Insufficient mineralization results in weak bones unable to withstand the stress of normal movement with bending of bones and fractures as a result. Folding fractures, secondary to nutritional deficiency, have previously been documented in amphibians [Green, 2001], reptiles [Knotek et al., 2003], birds [Phalen et al., 2005], and young children (i.e., torus fracture) [Allison 2008].

Limited data is available regarding specific nutritional needs for amphibians. *Leptodactylus fallax* is one of the few species in which data has been gathered. In one study, the stomach contents of 371 mountain chicken frogs were examined where crickets of the genus *Amphiacusta* were found in the gastric contents of 59% of all frogs [Brooks, 1982]. This accounted for 21% of all gastric items and 31% of total dry weight of food found in the stomach. Millipedes, coleopterans, and gastropods compromised large portions of the remaining stomach contents, which also included smaller frogs, a lizard, and a small mammal, either a young agouti (*Dasyprocta cristata)* or house mouse (*Mus musculus*). This type of prey consumption was described as opportunism and catholicity [Brooks, 1982]. And, even though it is known what adult *L. fallax* eat in the wild, it is unlikely such a variety of foods could be fed in captivity due to its complexity and difficulty in obtaining all of the items. Additionally, the nutritional content and bio-availability of each these food items are not known.

It is possible that *L. fallax* could be obtaining calcium and other minerals from another environmental source such as the water. Though *L. fallax* is considered a terrestrial frog it can be found soaking in water at night. Calcium and other minerals could be obtained from the water through the gastrointestinal tract or through skin absorption [Wood and Hillman, 2005]. Up to 80% of daily water uptake may occur through the pelvic patch [Wright, 2001], and it has been shown that frogs can absorb sodium from water through the pelvic patch [Parsons and Schwartz, 1991]. In calcium deficient water, bullfrog tadpoles develop skeletal deformities [Marshall et al., 1980] and leopard frogs may loose up to 0.1% daily of the total body calcium [Bentley, 1984].

The mineral content of the water in Dominica currently is unknown.

CONCLUSIONS

This study suggests we need to re-evaluate how *L. fallax* and other frog species are raised in captivity. Zoos and other institutions possessing captive bred *L. fallax* should examine their frogs for radiographic lesions consistent with MBD. Future studies including analyses of the various diets and water supplies used at each institution could be compared to assist in determining optimal conditions for the raising and management of captive frogs. Additionally, water samples from locations of wild population should be analyzed.

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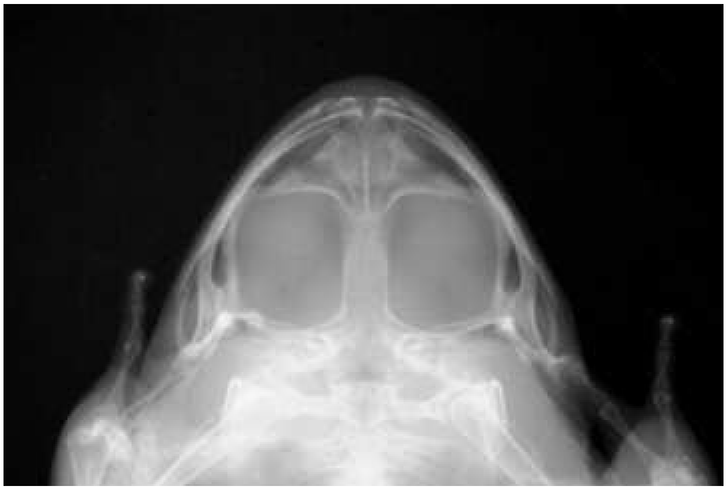
TABLE 1. Bones exhibiting folding fractures

Bones of the limbs	# Fractures per frog (Mean)	# Fractures per frog (Range)	% Fractures per single bone type
Femur	1.95	1-2	97.7%
Tibio-fibula	1.91	0-2	95.5%
Radio-ulna	0	0	0%
Humerus	0.45	0-2	22.7%
All bones of the limbs	4.32	2-6	54%

Wild caught frog with normal cortical density and no fractures.
223x283mm (72 x 72 DPI)

Captive born frog with cortical thinning and multiple folding fractures (arrows) of the hind limbs.
175x225mm (72 x 72 DPI)



Wild caught frog with well defined bones surrounding the orbits.
127x85mm (72 x 72 DPI)

Captive born frogs with bones surrounding the orbits which are misshapen and hard to define.
127x85mm (72 x 72 DPI)