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RESEARCH ARTICLE

Meeting Ultraviolet B Radiation Requirements of Amphibians in Captivity: A Case Study With Mountain Chicken Frogs (*Leptodactylus fallax*) and General Recommendations for Pre-Release Health Screening

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Conservation breeding programmes are a tool used to prevent amphibian extinctions. The husbandry requirements of amphibians are complex. Ongoing research is needed to ensure optimal management of those captive-bred animals destined, in particular, for reintroduction. The UV-B and vitamin D_3 requirements of amphibians are largely unknown. Metabolic bone disease has been reported in a number of species. These include the Critically Endangered mountain chicken frog (*Leptodactylus fallax*) reared in captivity on diets supplemented with a high-calcium multivitamin and mineral supplement containing vitamin D_3 but without UV-B provision. Captive-bred *L. fallax* being reared for reintroduction to Montserrat were provided with UV-B radiation from metamorphosis and were fed on insects supplemented with vitamins and minerals. Overlapping heat, light and UV-B gradients were provided, mimicking what we believe best represents the natural situation and thereby facilitated self-regulation of UV-B exposure. A subset of 10 frogs was periodically radiographed to assess skeletal health. Radiographic bone density and anatomical integrity appeared unremarkable when compared with a wild caught *L. fallax*. In addition to other routine health-screening, we recommend that radiography be performed to a structured schedule on a subset of all captive-bred and reared amphibians to assess skeletal health and to gauge the appropriateness of captive husbandry. We demonstrate here that, through the appropriate provision of a combination of both UV-B radiation and dietary supplementation, *L. fallax* can be bred and reared in captivity with healthy skeletal development. Zoo Biol. XX:XX–XX, 2014. © 2014 Wiley Periodicals, Inc.

Keywords: amphibian; conservation; husbandry; UV-B; *Leptodactylus fallax*

INTRODUCTION

Amphibians are in decline world-wide [Alford and Richards, 1999] with extinction rates up to 211 times the background rate [McCallum, 2007]. Conservation breeding programmes are one of the tools used to prevent amphibian extinctions [Griffiths and Pavajeau, 2008]. These programmes should aim to maintain genetically representative populations of amphibians in captivity for future population supplementation, reintroduction, or translocation programmes [Baker, 2007; Shishova et al., 2010; Browne and Figiel, 2011]. Amphibians are often cited as being ideal candidates for conservation breeding programmes due to their small size, high fecundity, and low maintenance requirements, and consequentially the cost effectiveness of such programmes when compared with programmes for other

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vertebrates [Bloxam and Tonge, 1995; Balmford et al., 1996; Jones, 2002].

It is now apparent that the captive husbandry requirements of amphibians are more complex than first thought [Browne et al., 2006; Antwis and Browne, 2009; King et al., 2011; Verschooren et al., 2011; Ogilvy et al., 2012; Dugas et al., 2013; Antwis et al., 2014]. Thus conservation breeding programmes could potentially produce maladapted amphibians that are unsuitable for release [Antwis and Browne, 2009; Ogilvy et al., 2012]. It is imperative that more research is undertaken to ensure that the husbandry of animals bred in captivity for the purpose of reintroduction is optimal.

Nutritional problems have been cited as a major barrier to the implementation of amphibian conservation breeding programmes [Antwis and Browne, 2009; Browne et al., 2009; King et al., 2011; Verschooren et al., 2011; Ogilvy et al., 2012; Dugas et al., 2013]. Vitamin D_3 deficiency is one such nutritional problem; vitamin D_3 plays a critical role in regulating calcium metabolism, as well as important roles in organ development, muscle contraction and the functioning of the immune and nervous systems [Wright and Whitaker, 2001]. In most vertebrates, vitamin D_3 is synthesized via exposure to the ultraviolet B radiation (UV-B) present in sunlight. Recent “metamorphs,” juveniles and adults of a number of nocturnal frog species have been observed basking in sunlight in the wild and diurnal retreat sites can also receive reflected UV-B [K. Bradfield, pers. obs; Tattersall et al., 2006; Michaels and Preziosi, 2013]. This may indicate that even nocturnal species may rely on exposure to UV-B radiation to synthesize vitamin D_3. Captive *Bombina orientalis* that were exposed to UV-B radiation had significantly higher serum vitamin D_3 levels than a control group, which did not have access to UV-B, indicating that this species is able to photobiosynthesize vitamin D_3 [Michaels et al., 2014]. Furthermore, this is supported by studies that have shown that captive-bred nocturnal frogs housed in enclosures without access to UV-B but that were fed a diet of insects supplemented with vitamins and minerals exhibited metabolic bone disease (MBD) [Gagliardo et al., 2010; King et al., 2011; Verschooren et al., 2011]. A study on *B. orientalis* indicated that routine dietary supplementation with calcium and vitamin D_3 using existing, commercially available supplements and standard dusting practices did not fully compensate for lack of UV-B exposure and that currently used gut loading diets may provide insufficient levels of calcium in feeder insects [Michaels et al., 2014]. In the laboratory *Trachycephalus resinifictrix* demonstrated improved growth and skeletal development when provided with UV-B [Verschooren et al., 2011].

One of the most common types of MBD is attributed to vitamin D_3 or calcium deficiency [Mader, 2006; Densmore and Green, 2007]. Vitamin D_3 (cholecalciferol) is a precursor to calcitriol, a hormone, which governs calcium metabolism. Vitamin D_3 can be obtained by its ingestion in food but in most vertebrates is produced endogenously by exposure to natural sunlight. Exposure of the skin to UV-B radiation from sunlight, specifically of UV-B wavelengths between 290 and 315 nm, causes the photolysis of provitamin D_3 (7-dehydrocholesterol) to previtamin D_3, which is then thermally isomerized into vitamin D_3. This is transported in the bloodstream to the liver and hydroxylated into calcediol (25-hydroxycholecalciferol), the circulating storage form of vitamin D_3. Calcitriol, the active hormone enabling calcium absorption from the gut, is synthesized from calcediol by the kidneys. Low vitamin D_3 levels result in insufficient calcitriol being produced and, as a consequence, serum calcium levels fall. The resulting hypocalcaemia stimulates production of parathyroid hormone, which in turn promotes calcium resorption from the bones in order to maintain serum calcium levels within the very small range compatible with life. Prolonged stimulation of the parathyroid glands results in Nutritional Secondary Hyperparathyroidism (NSHP) and continuous mobilization of calcium from the bone results in generalized skeletal decalcification [Holick, 2003].

A healthy skeletal system is of vital importance to amphibians. Compromised skeletal systems may have direct impacts on growth, prey acquisition, mobility, reproduction, and animal welfare. Amphibians, in particular anurans, have the ability to reserve calcium carbonate in endolymphatic sacs in the cranial cavity and spinal canal, which can be drawn upon in order to raise the levels of circulating ionized calcium [Stiffler, 1993a]. Amphibians are also able to store calcium carbonate as a layer below the skin in both adults and larvae [Bentley, 1984; Cheek et al., 1993]. During metamorphosis calcium from these stores can be utilized, along with calcium uptake across larval gills, for rapid ossification of bones [Pilkington and Simkiss, 1966; Bentley, 1984]. Calcium is also transferred from the female to the yolk during egg formation [Wysolmerski, 2002].

As a form of MBD, NSHP is diagnosed primarily through radiography [Klaphake, 2010]. Blood plasma studies may demonstrate imbalances in calcium to phosphorus ratios [Klaphake, 2010]. Frogs can recover from NSHP and bones can be re-calcified but skeletal deformities caused by NSHP may be permanent (Figs. 1 and 2).

Provision of appropriate UV-B is clearly important for the health of some captive amphibians [Antwis and Browne, 2009; Verschooren et al., 2011] but it is believed that there may be detrimental effects on growth and development if amphibians are exposed to higher levels of UV-B radiation than are found in that particular species' microhabitat [Licht, 2003]. To complicate matters, few studies have measured the exposure of wild amphibians to UV radiation in their specific micro-climates or how this may change as amphibians mature [Michaels and Preziosi, 2013]. As UV exposure parameters in the wild are largely unknown the provision of appropriate, naturalistic, gradients in captivity can be a challenge [Michaels and Preziosi, 2013]. Specialized lamps may be utilized to provide UV-B of the appropriate wavelengths to enable vitamin D_3 synthesis. Many zoos and aquaria are moving toward providing amphibians with free access, throughout their diurnal period,

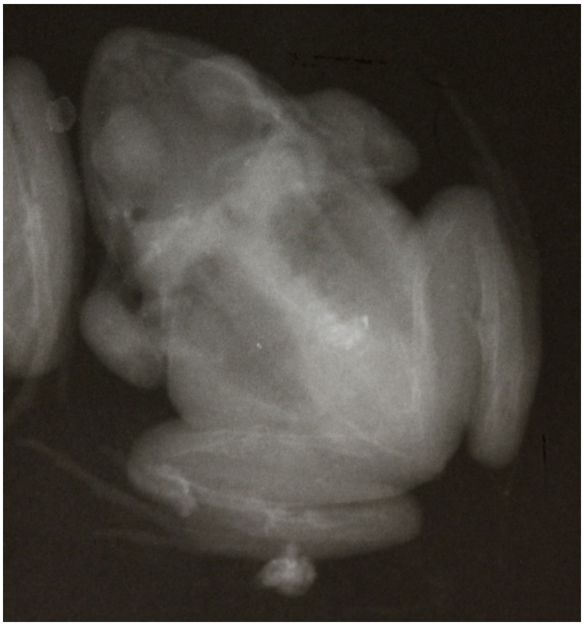

Fig. 1. Radiograph of a 5-month-old captive bred mountain chicken (*Leptodactylus fallax*) at Durrell Wildlife Conservation Trust raised without UV-B radiation exhibiting metabolic bone disease.

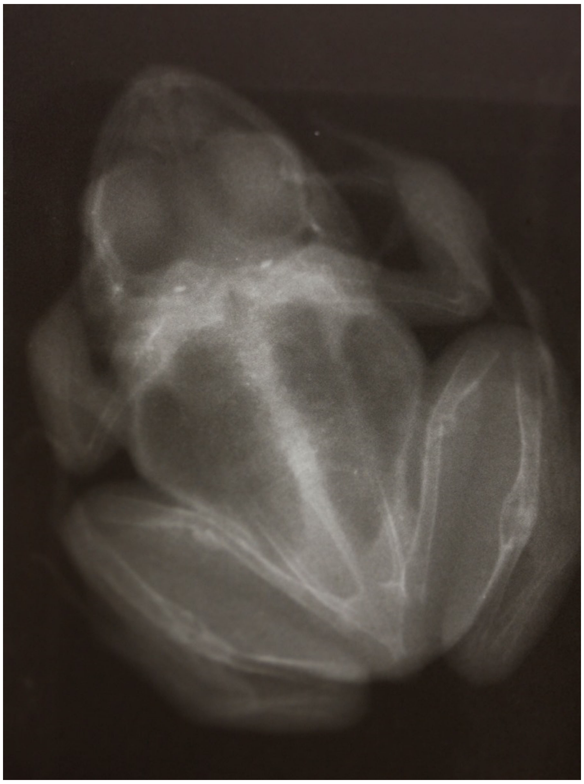

Fig. 2. Radiograph of an 8-month-old captive bred mountain chicken (*Leptodactylus fallax*) at Durrell Wildlife Conservation Trust initially reared without UV-B. UV-B was provided at 6 months of age and bone mineralization had improved but bone deformities remain.

to relatively low levels of UV-B radiation in a gradient within their microhabitat. Alternatively, a "boosting method" can be used in which amphibians are subjected to periodic short-term exposure to relatively high levels of UV-B radiation in a controlled setting [Verschooren et al., 2011].

Leptodactylus fallax is the largest native amphibian in the Caribbean region and is categorized as Critically Endangered on the IUCN Red List [Fa et al., 2010]. Currently the species is restricted to the islands of Dominica and Montserrat in the eastern Caribbean; it was formerly far more widespread, occurring on seven Eastern Caribbean islands [Schwartz and Henderson, 1991]. Historically, island extinctions came about through a combination of habitat loss and degradation, introduced predators and over-collection for food. More recently the only two extant island populations have been driven toward extinction by the emerging infectious disease amphibian chytridiomycosis [Malhotra et al., 2007].

In response to the population crashes resulting from the arrival of chytridiomycosis on Montserrat and Dominica, ex situ safety-net populations were established in 2009 at several institutions in Europe: ZSL London Zoo, Durrell Wildlife Conservation Trust, Chester Zoo, and Parken Zoo. A range-country facility was established in Dominica and has housed *L. fallax* since mid-2011 [Tapley et al., 2014]. The husbandry and breeding requirements of *L. fallax* are well-documented [Gibson and Buley, 2004]. Since 2011 a total of 121 mountain chickens bred at bio-secure facilities in Europe have been re-introduced to Montserrat [Adams et al., 2013]. King et al. [2011] reported MBD in captive-bred *L. fallax* of Dominican origin in the United States and attributed the disease to inadequate nutrition. The frogs were not provided with UV-B in this study.

The aim of this study was to gauge the effectiveness of current husbandry practices for rearing captive bred mountain chicken frogs, specifically the provision of UV-B and the supplementation of feeder insects, using skeletal growth and formation as proxy. Here, we present findings from first generation *L. fallax* bred in captivity from wild-caught parental stock at Durrell Wildlife Conservation Trust and ZSL London Zoo, the latter population being destined for reintroduction to Montserrat.

METHODS

In 2000, Durrell Wildlife Conservation Trust bred *L. fallax* in captivity for the first time. The metamorphs were reared in enclosures without UV-B radiation and were fed a diet of insects (*Gryllus bimaculatus and G. assimilis*) supplemented with a high-calcium multivitamin and mineral supplement containing vitamin D_3 (Nutrobal, Vetark Ltd., Winchester, UK). Several juvenile frogs were routinely radiographed 5 months post metamorphosis and were found to be exhibiting deformities of the femur, tibia and fibula indicative of MBD, a presumptive NSHP (Fig. 1). A husbandry change was made, namely the provision of UV-B radiation (Reptisun 5.0 UV-B, ZooMed Laboratories, Inc., San Luis Obispo, CA) Radiographs were repeated 3 months later. Bone calcification was deemed to have improved though the pre-existing deformities remained (Fig. 2).

In 2012 *L. fallax* were bred at ZSL London Zoo. A group of 76 frogs were reared with UV-B provision, from which a randomly assigned, known and identified, subset of 10 frogs was digitally radiographed every 3 months for a period of 9 months to assess bone formation and mineralization.

At metamorphosis 76 frogs were housed in four groups of 13 and two groups of 12 in 460 mm × 300 mm × 170 mm Faunariums (Exo Terra, Rolf C. Hagen (UK) Ltd., Castleford, UK). This set up had a single UV-B emitting 1,150 mm T5-HO UV-B fluorescent tube (D_3+ 12%UV-B Reptile Lamp, Arcadia Products plc, Redhill, UK) providing a gradient of UV-B radiation. This lamp was used in tandem with a full spectrum fluorescent tube (Reptisun 2.0 UV-B, ZooMed Laboratories Inc.) to provide broad spectrum lighting for the animals including a definite UV-B component in the range necessary for photobiosynthesis of vitamin D_3. Both were fitted with reflectors.

UV Index (UVI) readings were taken on a monthly basis with a Solarmeter 6.5 UV Index Meter (Solartech Inc., Harrison Township, MI). The UV Index is a unitless, international standard measurement of the strength of the ultraviolet radiation from the sun reaching the surface of the earth at a given point. To obtain the UV Index, an irradiance measurement is weighted according to the McKinlay–Diffey erythemal action spectrum, which takes into account the inverse relationship between wavelength and photobiological effectiveness. The Solarmeter 6.5 UV Index meter is designed to give a readout of the UV Index (UVI), since its sensitivity response follows the erythemal action spectrum. However, the meter's response is also sufficiently similar to the action spectrum for previtamin D conversion for it to provide a useful estimate of the potential of incident UV radiation for enabling cutaneous vitamin D synthesis. [Schmalwieser et al., 2006; Lindgren et al., 2008]. UVI gradients were measured through the Faunarium lids and ranged from UVI of 0–3.0 at the level of the frogs' dorsum.

Two months post metamorphosis the management changed and the 76 frogs were housed in two groups of 25 and one group of 26 in 2 m × 2 m × 3 m pens. All three groups were provided with an array of UV-B emitting lamps providing a gradient of UV-B radiation. The previous combination of lamps was used along with a UV-B emitting 300 watt mercury vapour lamp (Ultra-Vitalux, Osram GmbH, Augsburg, Germany). As before, UVI readings were taken on a monthly basis with a Solarmeter 6.5 UV Index Meter. UVI gradients again ranged from UVI 0–3.0 at the level of the frogs' dorsum. To maintain the intended UVI levels and gradient the placement of the lamps was adjusted, as required, on a monthly basis for the duration of the study.

For 2 months post-metamorphosis, frogs were fed ad libitum on a daily basis. Frogs were crickets (*G. bimaculatus* and *G. assimilis*), which had been fed daily on a variety of vegetables and offered spirulina powder for 24 hr prior to being fed out. Immediately prior to offering to the frogs the feeder insects were placed in a polythene bag and dusted with a high-calcium multivitamin and mineral supplement containing vitamin D_3 (Nutrobal). From 2 months post-metamorphosis onwards the frogs were fed ad libitum three times a week on *G. bimaculatus*, *G. assimilis*, and locusts (*Schistocerca gregaria*). As before, the feeder insects were fed and fed out to frogs as described above.

All frogs were percutaneously micro-chipped into the dorsal lymphatic sacs with passive integrated transponders at 5 months post-metamorphosis. Bearing in mind this species' documented propensity to develop MBD despite adequate dietary regimes [M. Goetz, pers. obs; J. Lopez, pers. obs; King et al., 2011] and that all 76 frogs were destined for release to the wild as part of a conservation program, it was deemed inappropriate to have a control group with sub-optimal or no UV-B provision.

A randomly assigned, known and identified, subset of 10 frogs from the group was digitally radiographed every 3 months for a period of 9 months to assess bone formation and mineralization. The radiograph images were processed using a Fuji Prima II computerized radiography system. The algorithm used to digitize the image was that of a small amphibian. The images for each study were saved and stored on an in-house PACS system. Frogs were aged 5 months post-metamorphosis at the date of the first radiograph. At the end of the study, the radiographs were reviewed and compared with published radiographs of a wild caught *L. fallax* [King et al., 2011] by the ZSL London Zoo veterinary team.

RESULTS

When compared with those of the frogs reared without UV-B provision (Figs. 1 and 2), the radiographs of the 10 *L. fallax* reared with UV-B, and on diets supplemented with a high-calcium multivitamin and mineral supplement containing vitamin D_3, demonstrated that all 10 frogs were skeletally unremarkable for the duration of the study period (Fig. 3). There was no evidence of ongoing or healed fractures or of bone deformation or re-modeling. Visually, bone density appeared normal in all frogs when compared with published radiographs of a wild caught *L. fallax* [King et al., 2011]. Cortical bone formation was assessed to be good (Fig. 3). One of the frogs from the subset of 10 was euthanazed at the end of the study. Skeletally, both in terms of mineralization and bone strength, it was deemed unremarkable but it had failed to grow in parallel with the others and would have been unsuitable for release to the wild. A post mortem examination and histopathological findings did not reveal the cause of the stunted growth exhibited in this specimen.

DISCUSSION

The UV-B and Vitamin D_3 requirements of amphibians are largely unknown. It is likely that different species will

Fig. 3. Radiographs of three 10-month-old captive bred mountain chickens (*Leptodactylus fallax*) that formed part of this study at ZSL London Zoo.

have different requirements due to their differing life histories and micro-habitats. The extent to which *L. fallax* actively bask in nature is not known but, on one occasion, two *L. fallax* of captive bred origin were observed basking in the early morning on Montserrat (Fig. 4). Frogs in zoo populations have been seen basking under UV-B emitting lights, both at ZSL London Zoo and Durrell Wildlife Conservation Trust [B. Tapley, K. Bradfield and M. Goetz pers. obs.], though this could have been primarily for thermoregulation. The captive frogs were frequently observed basking when the ambient temperature was within the range mountain chicken frogs experience in the wild. This suggests that basking in sunlight is a normal behavior in this species [B. Tapley, M. Goetz, K. Bradfield pers. obs.]. Even in light shade UV-B levels during daylight hours in the wild may be appreciable owing to the presence of diffused and reflected ultraviolet radiation from the sky [Turnbull et al., 2005].

Fig. 4. *Leptodactylus fallax* of captive bred origin basking in the early morning on Montserrat.

Wild amphibians have evolved in accordance with their environments. They will reconcile requirement and provision by self-regulating their exposure to heat, light and the associated UV radiation. We believe that the optimal UV levels for a species will be those in their natural habitat, specifically at the microhabitat level for each life-history stage. Where possible the UV-B microclimate throughout the year should be established through field studies and used to inform the captive management of the target amphibian species. In the absence of such data, one possible approach has been suggested by Ferguson et al. [2010] where the authors monitored the UV Index at the precise locations of 15 lizard and snake species in the field during their daily peak of activity. They found that the range of average voluntary UV-B exposures recorded for each species could be predicted by the basking behavior typical of each species. They arbitrarily assigned each species into one of four categories or UV-B "Zones." Zone 1 (range UVI 0–0.7; maximum recorded UVI 1.4) included crepuscular or shade-dwelling species; Zone 2 (range UVI 0.7–1.0; maximum recorded UVI 3.0) included daytime partial sun or occasional basking species; Zone 3 (range UVI 1.0–2.6; maximum recorded UVI 7.4) included full or partial sun basking species; Zone 4 (range UVI 2.6–3.5; maximum recorded UVI 9.5) included mid-day basking species. No amphibians were studied but the four Zones represent useful sub-divisions in the continuous spectrum of UV-B exposure levels in the wild. One could anticipate that one could extrapolate these categories to determine the UV exposure of within micro-habitats, which might be experienced by animals other than reptiles demonstrating similar microhabitat utilization. *Leptodactylus fallax* would thus be provisionally allocated into Zone 2 (occasional basking species); hence, in this study, they were provided with a UV-B gradient from zero to a maximum of UVI 3.0.

In order to mimic the situation in the wild, heat (infrared radiation), light and UV-B should be provided together (i.e., the highest levels of UV-B should be associated with the warmest areas of the enclosure, and the lowest levels with the coolest areas). Most importantly, all three must be provided as gradients in the enclosures for appropriate lengths of time each day in order to facilitate self-regulation, with retreats that provide deep shade and no direct UV radiation. If there are life-history stages that would, in the wild, only be exposed to low-level reflected or diffused UV-B, or none at all (e.g., eggs and tadpoles in tree holes or burrows), then care should be taken to replicate this situation in captivity.

The provision of appropriate UV-B radiation to captive amphibians is important for their health and proper development but this field is still in its infancy. We strongly advise caution in its provision as over-exposure can also cause harm. We believe that tolerances to UV-B radiation will be species-specific. More research is needed to determine optimal levels of UV-B exposure for the different amphibian species maintained in captivity. Excessive UV-B radiation or provision of inappropriate wavelengths can damage living cells (either by killing them or by damaging DNA and RNA). This can lead to relatively immediate or longer-term adverse effects [Gardiner et al., 2009]. Adverse effects recorded in amphibians as a result of exposure to unnaturally high (for the species in question) levels of UV-B radiation include cell death, retinal damage, immunosuppression and developmental mutations [Licht and Grant, 1997; Blaustein and Belden, 2003]. Nevertheless we strongly believe that UV-B has a role to play and that captive management of amphibians should not depend solely on dietary supplementation of vitamin D_3. Oral vitamin D_3 supplementation is all but straightforward. There is a paucity of data regarding the vitamin D_3 requirements of captive amphibians and there can be serious adverse effects through inadvertent under or over-administration. Even if a dose rate is known administering that dose, within therapeutic margins, to an individual can be a challenge. The provision of UV light circumvents this by facilitating self-regulated endogenous management of calcium homeostasis by each individual. However, the importance of understanding the potential issues associated with the use of artificial UV-B and how to minimize/avoid them therefore cannot be understated.

The existing IUCN reintroduction guidelines focus, quite rightly, on pathogens. As with pathogen screening, where the "source population" is used as the benchmark for assessment, the same population could also be used as the benchmark for physical and physiological parameters. As skeletal health is intrinsically linked with the frogs' ability to cope with new stresses encountered at the destination site its it would be beneficial if reintroduction guidelines incorporated the need to assess skeletal health prior to reintroduction, similarly using source animals as the standard point of reference. There would be a sequential optimization of welfare and increased assurances with the regard to the fitness for animals in these programmes should this be done.

CONCLUSIONS

Previous experience has shown that captive-bred *L. fallax* housed in enclosures without access to UV-B but fed a diet of insects supplemented with vitamins and minerals (including D_3) can succumb to MBD. Subsequent UV-B exposure in these frogs led to an improvement in radiographic bone density, with the caveat that this UV-B provision could not be quantified at the time (2000) as affordable hand-held UV meters were not available. All subsequent offspring bred were immediately and constantly provided with a UV-B source and none of these offspring exhibited any deformities and all showed good bone mineralization.

This study demonstrates that *L. fallax* can be bred and reared in captivity with demonstrably healthy skeletal development through using a combination of both UV-B radiation and dietary supplementation. We recommend that radiography be performed to a structured schedule on a subset of all captive-bred and reared amphibians in order to assess skeletal health and to gauge the appropriateness of the husbandry being applied. This is particularly important for amphibians in conservation breeding programmes where animals are intended for release to the wild in order to ensure that maladapted individuals are not used in reintroduction programmes.

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