UV-B, Vitamin D_3, and amphibian health and behaviour

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Browne RK, Verschooren E, Antwis RE, Vercaammen F. 2009. UV-B, Vitamin D_3. AArk Science and Research. http://portal.isis.org/partners/AARK/ResearchGuide/Amphibian%20zoo%20studies/Amphibian%20UV-B%20and%20Vitamin%20D3.pdf

For a review of the biology of Vitamin D_3 and UV-B metabolism in amphibians see; Antwis RE, Browne RK. 2009. Ultraviolet radiation and Vitamin D_3 in Amphibian Health, Behaviour, Diet and Conservation. Comparative Biochemistry and Physiology Part A154(2): 184-190.

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This document provides a short summary of the biological issues concerning studies of Vitamin D_3 and UV-B metabolism in amphibians. We also present some simple experimental designs.

There have been many effects on health of vertebrates in general attributed to poor Vitamin D_3 metabolism. However, only two effects have been recorded in amphibians. One is the loss of calcium from the skeleton and skeletal deformaties generically called nutritional metabolic bone disease (NMBD). The other, being the converse situation, is an overdose of Vitamin D_3 and the consequent elevated plasma calcium levels that cause excessive calcification of the skeleton and heart siezure. Generally in captivity a lack of dietary calcium or Vitamin D_3 is attributed to the common disorders of hunchback or rubbery legs. Often frogs not showing skeletal deformaties will still have little calcium in their bones, and perhaps reduced circulating calcium necessary for physiological processes.

There have been many effects on health of vertebrates in general attributed to poor Vitamin D_3 metabolism. However, only two effects have been recorded in amphibians. One is the loss of calcium from the skeleton and skeletal deformaties generically called nutritional metabolic bone disease (NMBD). The other, being the converse situation, an overdose of Vitamin D_3 and consequent elevated plasma calcium levels that causes calcification of the skeleton, increased myocardial excitability and heart seizure. In captivity generally a lack of dietary calcium or Vitamin D_3 is attributed to the common disorders of hunchback or rubbery legs. Often frogs not showing skeletal deformaties will still have little calcium in their bones, and perhaps in their plasma. Approximately 20% calcium loss in bones can be detected on radiographs and a loss of 35% can lead to rubebry legs.

A shortage of calcium in amphibian metabolism results in lack of calcification of the bones – called nutritional metabolic bone disease (NMBD), and other health problems including lethargy, poor growth, and possibly poor reproductive success. Both anabolism and catabolism are involved in Vitamin D_3 metabolism. Vitamin D_3 is required for the transport of calcium from the intestine into the blood stream. Vitamin D_3 can be acquired through the diet or by synthesis in the skin under the influence of UV-B. UV radiation is emitted by the sun as wavelengths ranging from 400 nanometres (nm) to 100 nm. This range is further subdivided into the sub-categories of UV-A (400–315 nm), UV-B (315–280 nm) and UV-C (280–100 nm). Only UV-A and UV-B solar radiation can naturally interact with biological systems, as wavelengths shorter than 290 nm are completely absorbed by the Earth's atmosphere (Antwis and Browne 2009).

Phosphorus is also an important component of bones and must also be included with calcium in the diet. Consequently, amphibians must have adequate calcium and phosphorus in their diet, and sufficient Vitamin D_3 must be supplied through the diet or by UV-B lights. For a discussion of the provision of dietary calcium and phosphorus see (Amphibian diet and nutrition).

Studies have shown that Dendrobatid species behaviourally regulate their exposure to UV-B; some aquatic amphibians can detect UV-B through their eyes (Deutschlander and Phillips 1995; La Touche and Kimeldorf 1979), and the ability to detect near UV-B radiation of 280 nm has been shown in frogs eyes (Govardovskii and Zueva 1974). More knowledge about UV-B behavioural regulation would enable more leeway in the optimal provision of UV-B in captivity, and may have consequences for amphibian conservation in nature.

UV-B can be provided by several types of lights. Some of these produce large amounts of UV-B along with a large amount of heat. These are often used for reptile. However, fluorescent lamps that run fairly cool are preferred for amphibians because of their generally low UV-B and temperature requirements. For information on the best lights to use see http://www.uvguide.co.uk/

Vitamin D_3 can be provided orally in feed, through supplemented live feed (crickets)(Li et al. 2009), through dietary supplementation of live feed (Li et al. 2009; bioteck.org 2009), through mixtures used directly for forced feeding, and as drops to the mouth (see Amphibian diet and nutrition). Vitamin D_3 can also be provided topically through the skin. Vitamin D_3 is fat soluble and in drops must be in a carrier like propylene glycol. Until further studies are completed for frogs we recommend a daily dose of 330 IU Vitamin D_3 per. kg per day given orally through drops. We consider it too difficult to give drops orally to very small frogs below 5 g, and even a measurable dose topically to frogs below 1.5 g.

Amphibians vary very highly in their exposure to UV-B. Amphibians include nocturnal, fossorial, or aquatic species with large variations in UV-B exposure. These vary from almost no exposure for fossorial amphibians or those that live in water more than 10 cm deep, to very high amounts for species that sun bask in during summer in temperate or tropical regions. The requirement for metabolic Vitamin D_3 and the ability of UV-B to promote the production of Vitamin D_3 in amphibians probably positively corresponds to their exposure to UV-B. Consequently, some species may have very low or no requirement for UV-B and low requirements for Vitamin D_3 from their diet. Others may have very high requirements. Knowing the patters and the amounts in the requirement for Vitamin D_3 and UV-B exposure between species from similar micro-habitats could help prevent hypervitaminosos.

However, even though we know that some species develop NMBD in captivity, even when fed crickets dusted with vitamin/mineral powder, there have been no formal studies to assess the needs of amphibians for UV-B and Vitamin D_3. To avoid the need for the use of continuous UV-B lighting some zoos including Chester Zoo (North of England Zoological Society) and Rotterdam Zoo, Netherlands, have used boost application of UV-B on Dendrobatid frogs. This consists of high levels of UV-B for short periods of 20 minutes monthly. Vitamin D_3 can be stored in the liver and with mammals has a half life of about two weeks.

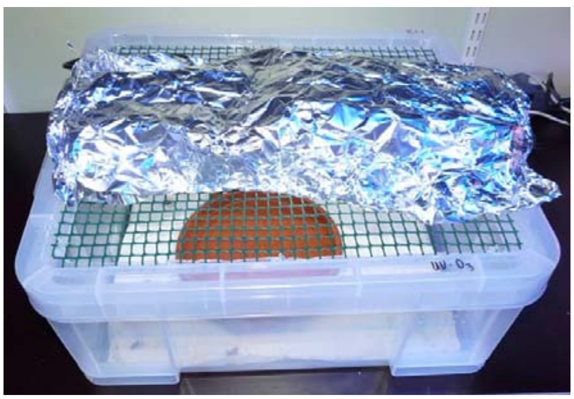
“The 'UV-B boost method was developed at Chester Zoo, UK, by Douglas Sherriff and Edwin Blake. Frogs are placed in an enclosure with a screen mesh lid that guarantees exposure of the frog to UV-B. A damp paper towel and cool room temperature reduce the chance of dehydration and overheating. If necessary lateral ventilation panels in the tank and a cooling fan can be used. This enclosure is placed beneath a UVB-emitting lamp – either an Osram Ultravitalux or a Zoologist Megaray – at a distance to directly achieve a UV-B level of 350-400μw/cm^2.

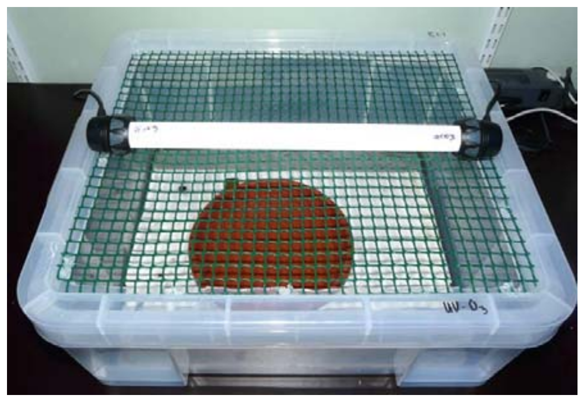
A finer mesh cover for very small frogs including (*Ranitomeya reticulatus* and *R.lamasi*) results in a reduction of UV-B exposure to about 300 μw/cm^2. Frogs are ‘boosted’ monthly for 20 min. Observation of the frogs throughout the boost and use of a timer is important to avoid problems with over-exposure. For example, a group of *R.reticulatus* was inadvertently boosted for 45 min. This resulted in superficial discolouration of the skin that healed within a fortnight. These frogs then went on to reproduce successfully (Gibson pers. com.).”

Studies of UV-B/Vitamin D_3 and amphibian health offer an ideal research focus for zoos, as very little is known about this subject that is so important to conservation breeding programs and the welfare of amphibians in captivity. Zoos, privates and other institutions can through pioneering studies of UV-B and Vitamin D_3 improve the health of captive amphibians, and provide basic knowledge to support the behavioural ecology and physiology of amphibians in nature. Studies of UV-B/Vitamin D_3 will also encourage studies of other nutrients and micro-nutrients.

Experimental design for studies of larval and adult amphibians.

We use plastic boxes (41 cm L x 34 cm W x 17 cm H) with a hole cut in the lid and covered with 1 x 1 cm x 1.7 mm plastic garden mesh. The bottom of the box is covered with paper towel and an 18 cm diameter x 2 cm depth water bowl provided. Paper towel was changed every two days and water replaced daily or with soiling. Lighting is provided by fluorescent lamps suspended over the mesh. For UV-B treatments strip lights are Zoo Med Reptisun 5.0 UVB (14W, 375mm/15”) or Zoo Med Reptisun 10.0 UVB (15W, 475mm/18”). An aluminum hood increases UV-B levels by 40%. UV-B levels were measured with a Zoo Med ST-6 Digital Ultraviolet Radiometer (range 280-325 nm, peak 290 nm, accuracy ± 10%). UV-B levels for Reptisun 5.0 in the box vary from 9-24 and with the Reptisun 10.0 from 23-50 µw cm^{-2}.





Above: A highly adapatable and robust habitat for testing UV-B is a plastic box with a hole cut in the lid and plastic garden mesh covering. A fluorescent light can be placed over the top of the mesh and then covered with analuminium foil cover. The aluminium cover reduces dispersion of the light and increase UV-V in the box by 40%.

Few studies have examined the behavioural sensitivity of adult amphibians to U-B radiation; however, Han et al. (2007) found UV-B avoidance behaviour in two Neotropical poison-dart frogs. Using a simple enclosure that provides UV-B or equivalent light without UV-B, and a web cam with a time lapse imaging system, researchers can with minimal effort reliably test UV-B avoidance behaviour in wide range of frog species. If the study is investigating behaviour the box can be partitioned with a vertical barrier, with a UV-B fluorescent tube either side, with Mylar blocking the UV-B on one side (Han et al. 2007).

Responses in UV-B/Vitamin D_3 studies include x-rays, weight and length, skin colouration and condition, and Vitamin D_3 levels. The inability to assay Vitamin D_3 levels except for plasma or whole body are restrictive. Blood can only be taken from larger amphibians In ectotherms, about 50% of the blood can be removed at one time. This is roughly 5% of the body mass. The smallest amphibian from which 0.2 ml whole blood needed for many assays can be sampled is 4 grams, and from which 0.5 ml whole blood can be removed weighs 10 grams (NWHC 2009). Tests for Vitamin D_3 (calcitriol) using radio imumo-assay require on drop of blood.

Left: Groups of many replicates can also be housed in similar arrangements. Juveniles of species that are sun baskers including Amazonian milk frogs (Trachycephalus resinifictrix)are easy to keep on a foam mat in a water bath in plastic containers with curtain mesh on top. This technique enables the housing of many replicates in a small area.

Amphibians physiologies and their needs for UV-B and Vitamin D_3 possibly vary dependent on their natural UV-B exposure. At Antwerp Zoo for research we are categorising anurans as receiving high, medium and low UV-B exposure in natuire. Green and Golden bell frogs (*Litoria aurea*), and some African reed frogs (*Hyperolius* spp.), that sunbask in the hot summer are considered high UV-B species. Amazonian milk frogs (*Trachycephalus resinifictrix*) males call from tree hollows but move during the day toward the canopy. The metamorph frogs have been observed to prefer exposure on foliage in captivity. They are related to the poison dart frogs and also produce a milky secretion that is probably discouraging to predators. Consequently, we regard *T*. *resinifictrix* as a medium UV-B requirement species in respect to natural UV-B exposure. Low UV-B exposure species are those that are crepuscular including tomato frogs (*Dyscophus antongilii*). How these categories relate to UV-B and Vitamin D_3 needs, and to behavour in respect to UV-B levels, are the subject of ongoing studies.

Right: Portable x-ray machines enable x-rays of very high resolution. This x-ray shows the early skeletal development of a tail bud T. resinifictrix only about 14 mm in snout-urostyle length.

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