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Review

Ultraviolet radiation and Vitamin D_3 in amphibian health, behaviour, diet and conservation

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ABSTRACT

Amphibians are currently suffering a period of mass extinction with approximately 20% of species under severe threat and more than 120 species already extinct. In light of this crisis there is an urgency to establish viable *ex situ* populations and also find the causes of *in situ* declines. The role of ultraviolet radiation and Vitamin D_3 in amphibian health directly influences both *ex situ* and *in situ* populations. Vitamin D_3 can be photosynthesised endogenously via UV-B radiation (UV-B), or acquired through the diet, and then metabolised to calcitriol the biologically active hormonal form. Although, there is a lack of literature concerning Vitamin D_3 requirements and calcitriol synthesis in amphibians, amphibians are likely to have similar Vitamin D_3 requirements and metabolic processes as other vertebrates due to the phylogenetically conservative nature of calcitriol biosynthesis. Deficiencies in calcitriol in amphibians result in nutritional metabolic bone disease (NMBD) and could compromise reproduction and immunity. However, excess biologically active UV radiation has also proven detrimental across all three amphibian life stages and therefore could impact both *in situ* and *ex situ* populations. Here we review the role and necessity of UV-B and calcitriol in amphibians and the potential for negative impacts due to excessive exposure to UV radiation. We also identify priorities for research that could provide critical information for maintaining healthy in *ex situ* and *in situ* populations of amphibians.

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1. Introduction

In light of global amphibian declines there is an increasing need to maintain *ex situ* populations both for conservation breeding programs and to alleviate excessive demands on wild populations for display, research and consumption (Stuart et al., 2004). However, common and often unrecognized problems in captive amphibians are weak skeletons because of nutritional metabolic bone disease as a result of inadequate calcium metabolism (Densmore and Green, 2007). Inadequate calcium metabolism in amphibians is often attributed to a Vitamin D_3 deficiency (Densmore and Green, 2007). Vitamin D_3 is a precursor to calcitriol, a hormone that is critical in the regulation of calcium metabolism and other metabolic functions (DeLuca, 2003; Holick, 2003). Vitamin D_3 can be acquired through diet or produced cutaneously in a process triggered by UV-B a component of biochemically active solar ultraviolet radiation (UV) (Webb, 2005; Chen et al., 2007). However, increased UV in the environment has also been suggested as a cause of amphibian declines (Alford and Richards, 1999). These have concerns have been fostered through increases in ambient UV levels caused by reductions in the ozone layer (Croteau et al., 2008). Although much is known about the UV-based synthesis and metabolism of Vitamin D_3 in mammals, and to a lesser extent in fish and reptiles, relatively little is known about the UV requirements and Vitamin D_3 metabolism of amphibians. Here we review the role of UV and Vitamin D_3 metabolism in amphibians whilst drawing comparisons with other classes of vertebrates; in particular Vitamin D_3 synthesis and, its metabolism to the biologically active form as calcitriol and its role in calcium homeostasis, and possible effects on health and reproduction.

2. UV radiation and Vitamin D_3 synthesis

2.1. UV radiation

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) (Fig. 1). 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 (Maclaughlin et al., 1982; DeLuca, 2003; Webb, 2005). UV-A and UV-B are both harmful when amphibians are exposed to high levels. However, UV-B is also beneficial to amphibians, and other vertebrates through initiating a series of chemical reactions in the epidermis that synthesise Vitamin D_3 and ultimately produce calcitriol and UV-A performs essential regulatory functions in Vitamin D3 regulation (Maclaughlin et al., 1982; Tian et al., 1996; Chen et al., 2007).

2.2. Synthesis of Vitamin D_3 and calcitriol via UV radiation

The biochemical pathway for cutaneous Vitamin D_3 synthesis is triggered by the photochemical lysing of provitamin D_3 by UV-B to produce previtamin D_3 the precursor of Vitamin D_3 (Holick, 2003;

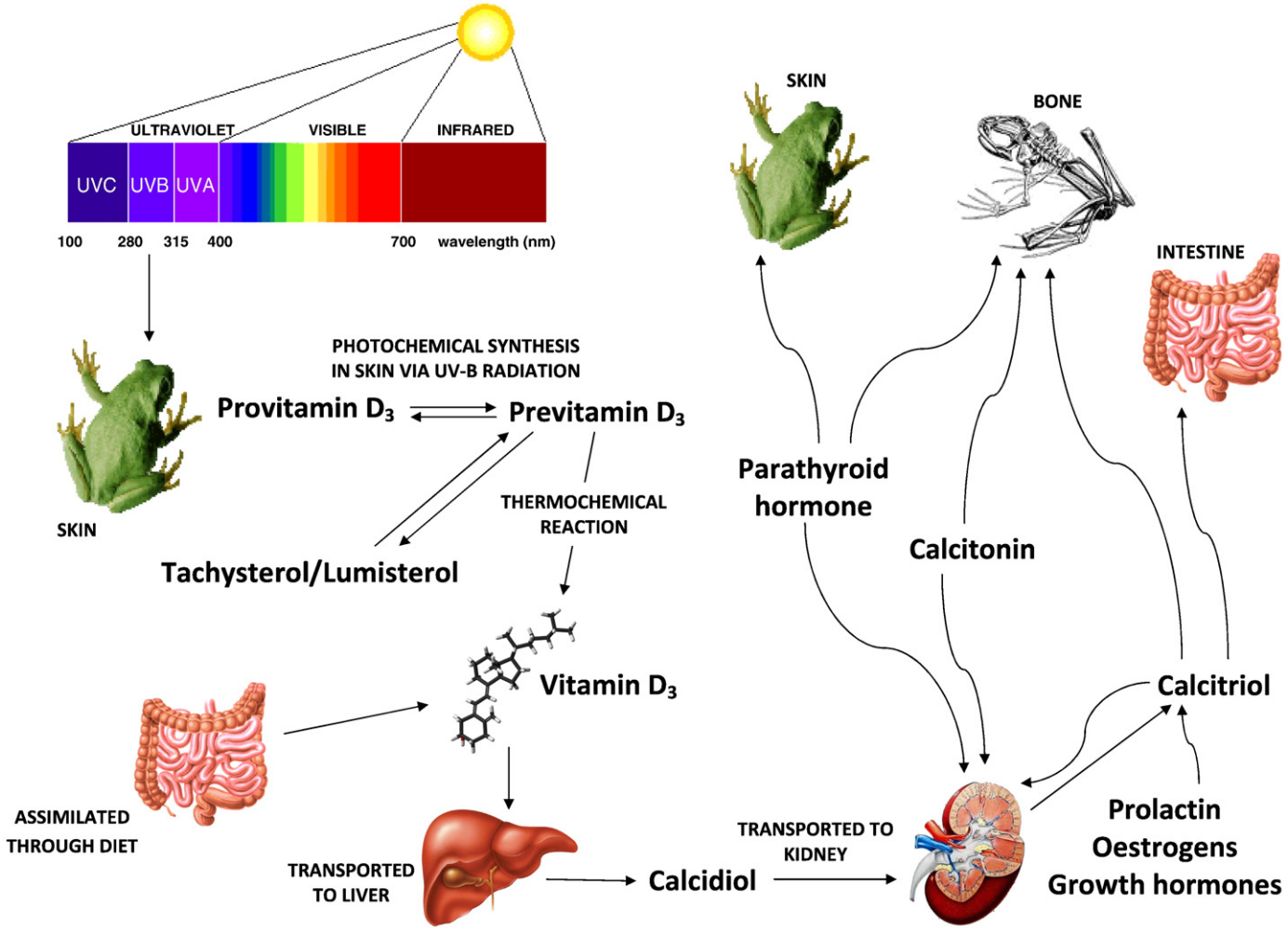

Fig. 1. The role of UV-B in the synthesis of calcitriol from its' hormonal precursors, including Vitamin D_3. The calcitriol synthesis pathway is indicated by straight arrows. The action of biologically active hormones on their respective target organs, or other hormones, is indicated by the curved arrows. Information collated from Stumpf et al. (1979), DeLuca (2003), Clark (1983), Webb (2005) and Holick (2003).

Webb, 2005). Both metabolites have been identified in epidermis of the bull frog (*Rana catesbeiana*), with varying concentrations between different epidermal tissues where the concentration of provitamin D_3 is more than double in the dorsal surface than in the ventral surface (Tian et al., 1996).

Previtamin D_3 can then be thermo-chemically isomerised into Vitamin D_3 (cholecalciferol) (Webb, 2005) with the rate of isomerisation increasing with increasing temperature (Lindgren et al. 2004). Alternatively, previtamin D_3 can be photo-isomerised into one of two inert compounds, tachysterol or lumisterol, or photochemically reverted back to provitamin D_3. This photochemical modulation of previtamin D_3 ensures excessive metabolic Vitamin D_3 levels do not accumulate (Webb, 2005). The conversion from previtamin D_3 to each of the compounds in the cycle is regulated by both UV-A and UV-B, so exposure to both UV ranges is necessary for adequate Vitamin D_3 metabolism (Maclaughlin et al., 1982). After synthesis, Vitamin D_3 is transported to the liver, where it is hydroxylated into calcidiol (25-hydroxycholecalciferol) then transported to the kidney where it is further hydroxylated into calcitriol (1, 25-dihydroxycholecalciferol) (Lindgren et al. 2004; Bjorn 2007). Fig. 1 summarizes the biochemical pathway of calcitriol synthesis, and its targets and associated hormones.

Calcitriol is the biologically active hormonal form of Vitamin D_3, and is responsible for maintaining calcium homeostasis through assimilation of calcium from the small intestine and calcium release from the kidney into the blood plasma, and calcitriol also acts within feedback pathways to maintain calcium homeostasis (Stiffler, 1993; Holick, 2003). Although literature on this subject frequently and incorrectly uses 'vitamin D,' and 'cholecalciferol' interchangeably with 'calcitriol,' in this review we shall henceforth solely and correctly use 'calcitriol' to pertain to the biologically active hormonal form of Vitamin D_3.

3. Calcium homeostasis in amphibians

3.1. Evolutionary role of ancestral amphibians in calcium homeostasis

Our understanding of calcium homeostasis in amphibians comes from studies in amphibian metabolism and biochemistry (e.g. Clark, 1983 and Stiffler, 1993), evidence from other vertebrate classes, and the evolution of ancestral amphibians. During their transition from freshwater to terrestrial environments ancestral amphibians developed unique physiological processes that enabled their transition through freshwater to terrestrial environments. During this transition there was a need for new mechanisms to regulate calcium metabolism for normal physiological function in hypocalcemic environments (Clark, 1983; Bentley, 1984). These mechanisms include the elimination of corpuscles of Stannius, found in saltwater fish and responsible for lowering blood serum calcium levels in both amphibians and other tetrapods. Conversely, the parathyroid hormone responsible for elevating calcium concentrations in the blood serum of tetrapods is not found in fish and therefore probably evolved first in ancestral amphibians (Clark, 1983).

3.2. Calcitriol in calcium homeostasis

The three main calcemic regulatory hormones in amphibians are calcitriol, parathyroid hormone and calcitonin (Stiffler, 1993). Details of these hormones origins and functions are listed in Table 1, although this review will mainly focus on calcitriol. Calcitriol, with other hormones, precisely regulates the concentration of calcium in blood plasma, the concurrent active uptake of calcium from stores including bone, and other diverse aspects of calcium metabolism (Stiffler, 1993; DeLuca, 2003; Webb, 2005). However, the primary function of calcitriol is the activation of calcium uptake from the intestine and glomular filtrate into the blood plasma (DeLuca, 2003).

3.2.1. Calcitriol and blood plasma calcium concentration

Calcium exists in blood plasma the form of free (ionised) calcium (Ca^{2+}) or as stabilised calcium within plasma proteins. This calcium in either form is then available for physiological processes or for bone mineralization (DeLuca, 2003). In many adult amphibians calcitriol acts to maintain calcium concentrations of between approximately 1.0 and 2.5 mmol l^{-1} in the blood plasma, with an approximate ratio of 50% ionised to stabilised calcium in the Northern leopard frog (*Rana pipiens*) (Stiffler, 1993). However, calcium concentration and the ratio of ionised to stabilised calcium can vary according to seasonal fluctuations and annual cycles of hibernation or aestivation (Stiffler, 1993). Blood calcium concentrations are also particularly variable during metamorphosis with large increases in concentration in response to demands for bone ossification (Stiffler, 1993). For example, blood plasma calcium concentrations are approximately 65% higher in post-metamorphosis *R. catesbeiana* (Ogura et al., 1975) and approximately 40% higher in tiger salamanders (*Ambystoma tigrinum*) (Stiffler, 1993).

3.2.2. Calcitriol and calcium stores

Calcium stores are important for regulating calcium concentration in blood plasma (Bentley, 1984; DeLuca, 2003). Calcitriol regulates for the preferential use of externally-sourced calcium prior to bone demineralisation and also for mobilising calcium stores, such as those in bone, into blood plasma during periods of calcium deficiency (DeLuca, 2003).

Amphibians also uniquely store calcium as calcium carbonate ($CaCO_3$) in specialised lymphatic sacs, and also as a layer below the skin in both adults and larvae (Bentley, 1984; Stiffler, 1993). During metamorphosis, calcium from these stores can be utilised along with calcium uptake across larval gills for rapid ossification of bones (Bentley, 1984). Calcium is also transferred from the female to the yolk

Table 1
Origin and function of the hormones involved in calcium metabolism in amphibians.

Hormone	Organ of production	Analogy to other vertebrates	Effect on $[Ca^{2+}]$ in blood plasma	Role in maintaining calcium homeostasis in amphibians	Associated hormones
Calcitriol	Kidney	Found in all vertebrates	Hypercalcemic	1. Initiates uptake of calcium from intestine and glomerular filtrate in kidney to blood plasma. 2. Stimulates osteoclasts to dissolve bone tissue during dietary calcium deficit thus releasing calcium ions into blood plasma.	Activated by pituitary hormone, oestrogens, growth hormone and prolactin
Parathyroid hormone	Parathyroid gland	Found only in tetrapods, excluding more primitive amphibians	Hypercalcemic	1. Mobilizes uptake of calcium from bones and reduces excretion in urine. 2. Works with calcitriol to stimulate calcium influx across the skin.	Calcitriol; antagonised by calcitonin
Calcitonin	Ultimobranchial bodies	Found in all vertebrates	Hypocalcemic	1. Initiates resorptive process for remineralisation of bone. 2. Encourages excretion via urine when calcium is in excess in the organism.	Antagonist to parathyroid hormone

Information collated from Clark (1983), Bentley (1984), Stiffler (1993) and DeLuca (2003).

during egg formation (Wysolmerski, 2002). Calcium is also important for other physiological and metabolic processes in amphibians including membrane stabilisation, muscle contraction, nerve transmission, cell secretion and enzyme regulation (Stiffler, 1993).

3.3. Nutritional metabolic bone disease

Nutritional metabolic bone disease (NMBD) develops in amphibians through deficiency or metabolic imbalances in calcitriol, calcium or phosphorus (Densmore and Green, 2007). The majority of cases of NMBD reported arise from inadequate dietary calcium or its assimilation or through deficiencies in calcitriol, causing the calcium stores in the skeleton to become depleted and the bones weakened (Densmore and Green, 2007). Phosphates are required for bone mineralization and are, along with calcium, absorbed at the intestine through the action of calcitriol (Bentley, 1984; DeLuca, 2003; Campbell, 2008). Alternatively, low exposure to UV-B can result in reduced calcitriol synthesis and poor uptake of calcium from the intestine (Holick, 2003; Densmore and Green, 2007). Hypervitaminosis of other vitamins such as Vitamin A may inhibit calcium uptake by antagonising calcitriol and encourage the development of NMBD (Johansson and Melhus, 2001; Densmore and Green, 2007).

4. Effects of UV-B exposure in amphibians

4.1. Ambient UV-B radiation

In global terms, ambient UV-B generally increases with altitude and latitude and UV-B levels are highest in the tropics between latitudes of 20° N and 20° S (McKenzie et al., 2007). UV-B levels are also affected by cloud cover, atmospheric pollutants, solar azimuth and reflection, and through absorption by the ozone layer (Licht and Grant, 1997). Although global ozone levels are subject to annual fluctuations, human-related depletion of the ozone layer in some regions has led to increased UV-B surface radiation, particularly at higher altitudes (Licht, 2003; McKenzie et al., 2007). Although ozone levels are expected to return to normal by the middle of the 22nd Century (United Nations of Environment Programme, 2008) UV-B exposure may currently be having undetected negative effects on amphibian survival (McKenzie et al., 2007). However, there is currently no evidence to indicate that increasing global levels of UV-B are having effects on amphibians at a population level (Corn, 2000). A study conducted by Middleton et al. (2001) used satellite data to map UV radiation over Central and South America and found significant increases in annual UV-B in eleven sites of amphibian decline between 1979 and 1988. However, the species in which declines were observed in these regions are forest-floor dwellers and unlikely to have much interaction with sunlight, indicating factors other than increased UV-B are causing the declines (Corn, 2005). Similarly, Davidson et al. (2002) found the patterns of decline for eight species of Canadian amphibians to be inconsistent with areas of higher elevation or more southerly latitude where higher stronger ambient UV radiation would be expected. It has also been suggested by Boone et al. (2003) that mortalities from ambient UV-B may increase the fitness of the amphibian population through natural selection, and that the mortalities are inconsequential when compared to mortalities resulting from predation and competition.

4.2. Excessive UV-B radiation

Although limited general conclusions of the effects of UV-B can be drawn across amphibian taxa, no information is available for defining the optimal exposure of amphibians to UV-B. Many studies of UV-B exposure on amphibians that have shown harmful effects on growth and development have tested higher levels of UV-B than are normally found in the species micro-habitat (Licht, 2003). Therefore, these studies cannot be used to realistically ascertain the effects *in situ* of UV-B on amphibians at a population level (Alford and Richards, 1999; Licht, 2003).

Nevertheless, many recent studies propose that amphibian pathologies are linked to excessive (higher than ambient) UV-B exposure. These studies suggest that increased intensity of UV-B at the Earth's surface due to ozone loss, or longer periods of exposure due to behavioural changes or habitat modification, could have affects on the survival, growth and development of amphibians during each life stage (e.g. Blaustein et al., 1994; Croteau et al., 2008). The range of pathologies in amphibians that are attributed to very high UV-B exposure include cell death, retinal damage, immunosuppression and developmental mutations (Licht and Grant, 1997; Blaustein and Belden, 2003), and disruption of the signalling pathways involving thyroid hormones which are particularly important for amphibian metamorphosis (Croteau et al., 2008). The majority of deleterious effects of UV-B exposure arise from DNA damage (Licht and Grant, 1997), however, all amphibians possess mechanisms for minimising UV-B damage, such as post-replication or excision repair of damaged DNA, the combined action of a photolyase enzyme with UV-A or visible light, and localised pigmentation to reduce UV penetration (Licht and Grant, 1997). These mechanisms have been identified in all life stages of amphibians (Rasanen et al., 2003), and a recent review by Licht (2003) into studies involving excessive UV-B irradiation concluded that these defence mechanisms are sufficient to cope with current and predicted increases in ambient UV-B levels.

4.3. UV-B radiation and amphibian life stages

4.3.1. Eggs and embryos

The exposure of amphibian eggs to UV-B in the aquatic environment is influenced by factors including UV-B levels, surface waves and reflected light, water depth, pH, temperature, water colour, dissolved carbon, along with phytoplankton and zooplankton concentrations (Booth and Morrow, 1997; Licht and Grant, 1997). Booth and Morrow (1997) conducted a meta-analysis on the available literature and reported the penetration of UV-B through various ecologically-differing bodies of water, including coastal waters and lakes. They concluded that UV-B may penetrate water from anywhere between 10 cm down to 10 m (measured to 1% of surface levels). A similar study by Morris et al. (1995) supported these values, and also indicated high dissolved organic carbon (DOC) was the main cofactor in UV-B attenuation. However, for the majority on aquatic sites with coloured water, surface level UV-B will not penetrate beyond the top few centimetres with nearly all UV-B attenuated at a depth of 10 cm (Morris et al., 1995; Booth and Morrow, 1997). For example, in a marsh with moderate levels of DOC the surface level of UV-B radiation was halved at 2 cm deep and was only 2% of initial values at 5 cm depth (Booth and Morrow, 1997).

The influence that UV-B exposure will have on developing amphibian embryos is dependent on a number of abiotic and biotic factors. The oviposition site is critical in for determining the level of exposure a spawn of eggs will receive (Licht and Grant, 1997). Eggs spawned nearer or on the water surface, in direct sunlight, in cooler or clearer water, or in water with a lower dissolved carbon content will be exposed to higher levels of UV-B (Licht, 2003). Eggs spawned in spring, when ozone levels are lowest, would be expected to be exposed to more UV-B irradiation, as would those spawned in direct sunlight (Licht and Grant, 1997). Spawn conformity may influence relative UV-B exposure of eggs where the shape and size of egg distribution results in different exposure of eggs to UV-B; for example, a string of eggs versus a clump of eggs (Licht and Grant, 1997).

Amphibian eggs have several mechanisms for reducing potential for UV-induced damage. Eggs and embryos produce the DNA repairing enzyme photolyase and melanin pigmentation which helps to protect them from UV-B damage (Licht, 2003). The egg and early developing embryo are also surrounded by a gel capsule that may reduce UV-B penetration in some species (Licht and Grant, 1997; Rasanen et al.,

2003). Exposure of an embryo to UV-B may also decrease the UV-B susceptibility of the resultant tadpole; the larvae of both the mole salamander (*A. talpoideum*) and the spotted salamander (*A. maculatum*) have shown to exhibit greater mortality when irradiated with naturally occurring and enhanced levels of UV-B light if not exposed during the embryonic stages (Calfee et al., 2006).

4.3.2. Larvae

The level of UV-B that can penetrate water and interact with larvae is affected by similar factors as for embryos, and similarly pigmentation offers larvae some protection from UV-B radiation and also photolyase can repair damaged DNA (Licht, 2003). However, amphibian larvae have the advantage of motility over embryos and it has been suggested that they may select different micro-habitats with different levels of exposure to UV-B (Licht, 2003). For example, larvae may select shallow warm water for thermoregulation or for foraging, thus increasing UV-B exposure. In contrast, cryptic or nocturnal behaviours will reduce UV-B exposure, as do avoidance behaviours including micro-habitat selection such as vegetative shading or aquatic immersion (Blaustein and Belden, 2003; Licht, 2003). However, physiological mediation of avoidance or selective behaviour depends on amphibians' ability to detect UV-B and exposure levels. Bancroft et al. (2008) found that tadpoles of three frog species (western toad (*Bufo boreas*), Pacific tree frog (*Pseudacris regilla*) and Cascade's frog (*Rana cascadae*)) and of the long-toed salamander (*A. macrodactylum*) do not intentionally avoid UV-B exposure but seek warmer temperatures regardless of UV-B levels. Similarly Belden et al. (2003) found that tadpoles of *R. cascadae in situ* do not intentionally avoid UV-B exposure, and do not exhibit a hormonal response when exposed to UV-B, showing that ambient levels of UV-B had no discernable effect on these species.

The effects of UV-B exposure on tadpoles has been shown to differ between species and with intensity. Grant and Licht (1995) showed that ecologically relevant doses of UV-B did not affect developmental period, duration of metamorphic climax, or mass at metamorphosis in *B. americanus*, *Hyla versicolor*, or *R. sylvatica*. However, a study by Bridges and Boone (2002) found that survival to metamorphosis by the southern leopard frog (*R. sphenocephala*) tadpoles increased by about 20% as subsurface UV-B intensity increased. Nevertheless, artificially high levels of UV-B exposure to larvae can cause spine deformation, reduced development rate, reduced predator avoidance and increased mortality (Grant and Licht, 1995; Licht and Grant, 1997; Kats et al., 2000). A major problem with many studies is the inability to extrapolate lab-based embryonic and larval studies to an ecological level to assess the effects of UV-B on amphibian populations (Alford and Richards, 1999; Corn, 2005).

4.3.3. Adults

There is a paucity of literature regarding the effects of UV-B on adult amphibians, particularly at the ambient levels in micro-habitats. Nevertheless, Zavanella and Losa (1981) showed that high levels of UV-B irradiation of the European crested newt (*Triturus cristatus carnifex*) resulted in significant skin damage and elevated mortality. However, adult amphibians are the most capable of the embryo, larvae and adult life stages at micro-habitat selection through their increased mobility, behavioral sophistication, and potential range of habitat types. Therefore, in nature adults of many species should be able to avoid excessive UV-B exposure more easily than either larvae or embryos (Blaustein and Belden, 2003). A study by Han et al. (2007) showed that two species of diurnal poison dart frogs, *Dendrobates pumilio* and *D. auratus*, chose shelter where UV-B is filtered or reduced. However, responses to UV levels and sunlight are difficult to discriminate and can confound studies. Colouration provides a mechanism to reduce UV-B penetration, where Roth et al. (1996) found that the wood frog (*R. sylvatica*) darken on exposure to UV-B, indicating the activation of protective pigments in the skin.

5. UV-B and calcitriol requirements of amphibians

In terrestrial vertebrates, a balance exists between the beneficial and detrimental effects of exposure to UV-B radiation. How does this balance vary within the amphibian class? How much exposure to UV-B is needed for calcitriol synthesis, and is dietary Vitamin D_3 alone sufficient to provide calcitriol for an amphibian? Although there is a paucity of studies of the UV-B and dietary Vitamin D_3 requirements of amphibians, various studies have been conducted for other vertebrates that may offer insight into their comparative requirements. (e.g. Carman et al., 2000; Ferguson et al., 2003; Ferguson et al., 2005; Acierno et al., 2006).

Carman et al. (2000) compared the *in vitro* percentage photoconversion in skin of provitamin D_3 to previtamin D_3 under UV-B radiation of the diurnal Texas spiny lizard (*Sceloporus olivaceous*) and the crepuscular house gecko (*Hemidactylus turcicus*). The efficiency of photoconversion inversely corresponded with natural UV-B exposure, with efficiency eight times higher in the crepuscular species, and similar results have been shown in *Anolis* lizards and bats (Ferguson

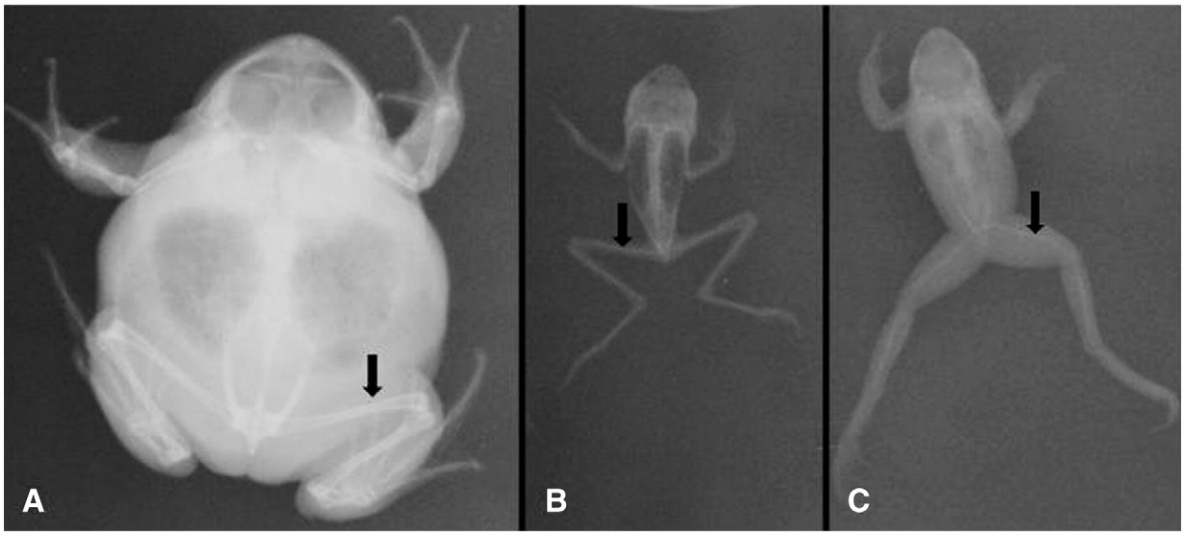

Fig. 2. X-ray images of individuals of three species of anura; **A** *Dyscophus antongilii*; **B** *Trachycephalus resinifictrix*; **C** *Litoria aurea*. All individuals were raised on the same Vitamin D_3-supplemented diet in the absence of UV-B radiation. The cortical region of the bones is indicated by the arrows, in which the burrowing *D. antongilii* shows much greater bone ossification than either *T. resinifictrix* or *L. aurea*, whose behaviour in the wild would allow greater UV-B exposure.

et al., 2005; Southworth, 2004). This evidence shows that some reptiles and mammals from low light UV-B environments have greater photoconversion of provitamin D_3 to previtamin D_3, and this may also prove to be the case in amphibians.

Amphibian species would be expected to show a very wide range of tolerances and needs for UV-B exposure depending on their habitat and behaviour. For example, the green and golden bell frog (*Litoria aurea*; Browne and Edwards, 2003) and the Amazonian milk frog (*Trachycephalus resinifictrix*), whose basking behaviours expose them to moderate or high levels of radiation, may require higher levels of UV-B radiation than a cryptic nocturnal species such as the Madagascan tomato frog (*Dyscophus antongilii*). In support of this proposal, X-ray images of captive amphibians that received supplementary Vitamin D_3 and no exposure to UV-B have shown *D. antongilii* to have greater bone ossification than *L. aurea* or *T. resinifictrix* (Fig. 2). Some amphibians such as fossorial caecilians have zero or very low natural exposure to sunlight and may be very sensitive to UV-B. This variation between species in sensitivity to UV-B also extends to amphibian embryos. Palen et al. (2005) conducted a study into the respective sensitivities of the embryos for four amphibian species (*R. cascadae*, *P. regilla*, *A. macrodactylum*, and the northwestern salamander (*A. gracile*)) to UV-B exposure by oviposition site and found those with an *in situ* oviposition with lower UV-B exposure displayed increased sensitivity to UV-B.

6. Vitamin D_3 and UV-B in captive amphibians

Some species of amphibians will successfully reproduce in captivity without the provision of UV-B including widely kept genera such as the diurnal Dendrobatids (*Dendrobates spp.*), and nocturnal arboreal species such as the Amazonian milk frog (*T. resinifictrix*) (pers.obs.). However, it is well known that many amphibian species are difficult to breed in captivity, and this could be attributed to an absence of UV-B and/or deficiency of dietary Vitamin D_3. For example this is seen in reptiles when exposure to excessively high or excessively low UV-B radiation results in reduced reproduction and fecundity in the panther chameleon (*Furcifer pardalis*) (Ferguson et al., 2002), and this may also be true for amphibians. Likewise in other reptiles, an artificial UV-B source has been shown to be necessary for maintaining health and reproduction in a wide variety of genera including the red-eared slider turtle (*Trachemys scripta elegans*) (Acierno et al., 2006), green iguanas (*Iguana iguana*) (Hibma, 2004), and panther chameleons (*F. pardalis*) (Ferguson et al., 2002), and could also be true for amphibians. Improved health and reproduction of amphibians has been widely reported through the 'boost protocol' application of high levels of UV-B for short periods of hours at long intervals of weeks (Gibson R. and Garcia G. pers com).

Dietary supplementation is an appealing method for providing Vitamin D_3 to captive amphibians due to its convenience and low cost. Dietary supplementation is achieved through the provision of Vitamin D_3 by topical powders or through dietary supplementation of feeder insects. However, topical powders are rapidly lost after application, thus reducing the supplementation and rendering the provision unquantifiable (Li et al., 2009). An additional uncertainty with dietary supplementation is that species specific Vitamin D_3 requirements are unknown, and supplements typically contain a wide range of vitamins and minerals that may result in hypo- or hypervitaminosis (Li et al., 2009). Consequently, the provision of UV-B through lighting is often considered an attractive alternative to dietary supplementation. However, the provision of UV-B lighting is costly and space consuming, and may be ineffective or even harmful without adequate knowledge of correct levels to fulfil the requirements of a particular species.

7. Conclusions and future directions

Calcitriol is the biologically active form of Vitamin D_3 and is a critical hormone for the correct functioning of calcium homeostasis in amphibians. A deficiency in calcitriol is known to cause nutritional metabolic bone disease, and similarly calcitriol imbalances may affect other processes including reproduction. With some species in conservation breeding programs the positive metabolic effects of UV-B in producing Vitamin D_3, or the provision of Vitamin D_3 through diet, may be necessary to maintain health. Consequently, a primary challenge in captive amphibians is the provision of adequate levels of either dietary Vitamin D_3 or UV-B lighting to enable synthesis of calcitriol in the interests of maintaining healthy populations. Because of difficulties in providing UV-B radiation to captive amphibians in many circumstances the efficacy of the application of 'boost protocols' should be further researched.

However a paucity of research in this field requires further studies to quantify the effects of calcitriol metabolism and deficiency in amphibians. Metabolism and transport of calcitriol are co-affected by lipids and other vitamins and hormones and further studies are also needed to elucidate these interactions. The development of benign methods to accurately assess the circulating levels of Vitamin D_3 or calcitriol, and bone density, are essential to studies of nutritional metabolic bone disease and pathologies in many captive amphibian models particularly those in zoos captive as current methods require euthanasia for samples or for bone ash measurements. Potential benign methods include analysis of urine or skin extrusions or the development of image analysis of digital X-rays for bone density measurements.

Concurrent with the uncertainties concerning the provision of Vitamin D_3 for amphibian health are the possible effects of excessive UV-B exposure. Detrimental effects of excessive exposure of UV-B on amphibian populations in nature have been postulated as a factor in amphibian declines. Continued research of Vitamin D_3 metabolism, calcitriol, and UV-B may prove crucial for successful reintroduction programs for threatened species, especially in light of globally changing UV levels. Many basic husbandry techniques for amphibians are uncertain, including UV-B requirements and the value of Vitamin D_3 provision through diet. Consequently, the challenges presented by elucidating Vitamin D_3 metabolism, the interface of both *ex situ* and *in situ* studies, and the development of increasingly benign methodologies provide an exciting field of research critical for amphibian conservation.

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