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The Effects of Ultraviolet Radiation on the Biology of Amphibians[1]

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SYNOPSIS. **Potential causes for the global decline of amphibians include habitat loss, disease, environmental contaminants and climate changes. The diminishing ozone layer and consequent increase of ultraviolet-B radiation reaching the earth's surface has been hypothesized to be a mortality factor, especially in habitats otherwise undisturbed. We discuss the fundamental physics of UV and types of biological damage after exposure. A deleterious change in DNA, especially the production of pyrimidine dimers, is a main effect of UVB exposure. Damaged DNA can be repaired by enzymes such as photolyase when organisms are irradiated with UVA or visible light. We review studies including laboratory and field approaches on damage to amphibians from UVB exposure. Field studies in which embryos were exposed to natural sunlight or sunlight with UVB removed have shown conflicting results: some show increased embryonic mortality after UVB exposure, whereas others show that current levels of UVB are not detrimental to amphibian embryos. The abiotic factors such as water depth, water color, and dissolved organic content of aquatic oviposition sites effectively reduces UVB penetration through water and reduces exposure to UVB of all life history stages. Biotic factors such as jelly capsules around eggs, melanin pigmentation of eggs, and color of larvae and metamorphosed forms further reduce effectiveness of UVB penetration. We suggest areas of future research to test the hypothesis of the causal connection between current UVB levels and amphibian decline.**

INTRODUCTION

A number of causal agents have been suggested for the apparent global decline of amphibians (Barinaga, 1990; Blaustein and Wake, 1990; Wake, 1991). Loss from habitat destruction and removal of breeding sites are straightforward (Moyle, 1973; Cooke and Ferguson, 1976; Johnson, 1992), but more indirect causes are difficult to ascertain and confirm. Environmental contaminants can affect the biology of amphibians: eggs, larvae, and metamorphosed forms of anurans and urodeles are adversely affected by acid precipitation (Dunson *et al.*, 1992; Sadinski and Dunson, 1992; Grant and Licht, 1993), heavy metals (Ireland, 1977; Birge *et al.*, 1979), and pesticides (Johnson, 1980; Vardia *et al.*, 1984; Licht, 1985). Outbreaks of disease such as redleg (*Aeromonas hydrophila*) or fungus (*Saprolegnia*) can be agents of mass mortality in at least restricted populations (Nyman, 1986; Bradford, 1991; Carey, 1993), and the likelihood of such pathogens causing death over wider geographic areas is a possibility (Kagarise Sherman and Morton, 1993). Climate disturbance in the form of moisture and temperature relations has been hypothesized as responsible for declines in some localized species, like *Bufo periglenes,* living in relatively restricted, specialized habitats (Pounds and Crump, 1994). In places where definite extinctions have not occurred, the reality of amphibian decline for some species has been questioned as perhaps being only a phenomenon related to natural fluctuations in numbers as a function of moisture-drought cycles (Pechmann *et al.*, 1991).

The thinning of the ozone layer and consequent increase of ultraviolet radiation (UV) reaching the earth's surface has focused attention on UV as a potential global agent responsible for decline, especially for species in habitats not obviously affected by other causes of mortality. Other causes for

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declines of nocturnal amphibians, those in shaded rain forest habitats, and those diminishing in numbers prior to the mid-1980s and UVB increase, remain conjectural. The effects of UV on the life history stages of amphibians is the subject of this report.

DISCUSSION

Physical aspects of UV

The physics and biological effects of UV on cells and uni- and multi-cellular organisms can be found in several comprehensive reviews (Koller, 1960; Jagger, 1985). Only the fundamentals related to potential danger to amphibians will be considered here. Ultraviolet radiation is a component of solar radiation and can be divided into 3 forms based on wavelength. Far-UV is UVC (<280 nm), and is completely absorbed by atmospheric gases and does not reach the earth's surface. Mid-UV or UVB (280–320 nm), does reach ground level, but the amount is considerably reduced by the layer of stratospheric ozone between 10 and 40 km above the earth. Near-UV or UVA (320–400 nm), passes fairly unimpeded through ozone to reach the earth's surface.

The solar flux at the top of the atmosphere is 1376 W/m^2 and about 8% of this is UV, of which 1.5% is in the UVB range (Allen, 1994). The amount of UVB reaching the earth's surface is affected by a number of factors including ozone, atmospheric pollutants, cloud cover, albedo, sunspot activity, latitude, angle of sun and others (Bjorn, 1989; Wellburn, 1994). Fluctuation of the ozone layer has a considerable effect, and depletion of the ozone layer by 5% can result in approximately a 10% increase of UVB at the earth's surface (Allen, 1994; Wellburn, 1994). Due to man-made chlorinated fluorocarbons and their effect on destroying ozone, estimates are that UVB will increase by 7% per decade over the northern hemisphere. A number of studies have shown changing trends in UVB (Madronich, 1992; Kerr and McElroy, 1993), but there is controversy over the magnitude and predictability of such changes, as well as over what geographic areas the changes will occur (Scotto *et al.*, 1988; Kerr and McElroy, 1994; Michaels *et al.*, 1994).

Biological effectiveness of UV

Ultraviolet-C is extremely disruptive to biological systems with UVB less so; UVA is not typically associated with extensive biological injury (Caldwell, 1971; Jagger, 1985; Wellburn, 1994). Erythema (sunburn) in exposed human skin is most effectively produced by UVB. As well, immunosuppression and carcinogenic and mutagenic effects have been clearly established in a wide variety of organisms (Setlow, 1974; Black and Chan, 1977; Jagger, 1985). The main target appears to be DNA and the formation of intrastrand pyrimidine dimers between adjacent thymine residues (Wacker *et al.*, 1961; Setlow *et al.*, 1965; Jagger, 1985). There are three mechanisms of repair of altered DNA: (1) excision repair, involving the selective removal of thymine dimers (Setlow and Carrier, 1964), (2) photoreactivation and the specific repair of pyrimidine dimers by photoreactivating enzymes (Blum *et al.*, 1949; Setlow and Setlow, 1963; Hill, 1965), and (3) postreplication repair (Lehmann, 1974). Irradiating organisms with UVB simultaneously, or immediately after, with either UVA or visible light (blue wavelengths most effective), will facilitate photorecovery. Photoreactivation and the presence of a specific enzyme, photolyase, has been found in a wide variety of organisms (Blum et al., 1949), with the evolutionary implication that it is a conserved feature and a general mechanism for protection against UV damage (Blum and Matthews, 1952).

Recently a photoreactivating enzyme that specifically repairs UV-induced (6–4) photoproducts was found in *Drosophila* (Todo *et al.*, 1993). Cyclobutane pyrimidine dimers and pyrimidine (6–4) pyrimidone photoproducts are the two recognized classes of deleterious DNA photoproducts with the former repaired by photolyase. The ubiquity of the newly discovered photorepair enzyme, from a phylogenetic perspective, has yet to be determined (Todo *et al.*, 1993).

Studies with amphibians

Ultraviolet-A is not as potent and biological damage is not as likely as from the other

two forms of UV. The ecological relevance of UVC is negligible because the energy does not reach the earth's surface and UVB is the wavelength of biological concern. Nevertheless, damage from UVC is of interest because it is similar to that of UVB (Jagger, 1985).

Studies with UVC typically use mercury arc lamps that generate radiation at peaks between 254 and 280 nm. The radiation readily deactivates the egg nucleus and is a useful tool in nuclear transplantation experiments (Gurdon, 1960). The irradiation of newly fertilized eggs affects the neural induction system and dorso-anterior structures in embryos (Scharf and Gerhart, 1980; Youn and Malacinski, 1980; Elinson and Pasceri, 1989). Irradiation of *Rana pipiens* eggs with a quartz mercury lamp at a distance of 25 cm for 1–2 minutes daily yielded embryos with abnormalities including twisting of the neural ridges, curvature of the medullary plate, and cephalic enlargement (Higgins and Sheard, 1926). Beudt (1930) found similar results with embryos of *Rana temporaria.*

In several of these studies (Higgins and Sheard, 1926; Beudt, 1930; and Gurdon, 1960), a significant discovery was that the jelly coat surrounding the ova absorbed UV and, indeed, the jelly would liquefy and dissolve after exposure to sufficient levels of irradiation. Both the jelly itself and the vitelline membrane surrounding the ovum proper are important in absorption of radiation and both Beudt (1930) and Gurdon (1960) indicated that the amount of radiation needed to cause embryonic deformities depends on the size and thickness of the surrounding jelly.

Few laboratory experiments have been done using UVB, the wavelength of ecological relevance. Zavanella and Losa (1981) irradiated adult newts, *Triturus cristatus carnifex,* with sunlamps in the spectrum of 275–350 nm. Animals received a total fluence of 1.3×10^5 J/m^2 in either low or high single flux of three irradiations per week. The tests continued for 7 months. Animals irradiated 30 min at each time showed the highest mortality, but mortality was low and did not differ between groups irradiated at 5 or 15 min. A few animals developed skin lesions, but most showed increased skin sloughing and cornified epidermis. Histological examination indicated a variety of skin abnormalities. Epidermal hyperplasia was the most notable feature in animals irradiated at low fluences with dermal fibrosis and epidermal atrophy most characteristic of animals treated at higher total fluences.

Worrest and Kimeldorf (1975, 1976) exposed embryos of *Bufo boreas* to enhanced UVB (290–315nm) administered by sunlight simulating fluorescent lamps. Tadpoles exposed daily to enhanced UVB displayed abnormal development of presumptive cornea, epidermal hyperplasia, curvature of the spine ("lordosis") and increased mortality. Daily exposures ranged from 7.5 kJ/m^2 to 41 kJ/m^2 and severity of effects increased with dose.

We (Grant and Licht, 1995) found no effect of UVA on embryos of *Rana sylvatica* or tadpoles of *Hyla versicolor, Rana clamitans,* and *R. sylvatica.* We also tested the effects of artificially high UVB (using unshielded lamps at spectral peak of 302nm) and reduced levels (lamps shielded with cellulose acetate) on one or more stages (embryos, tadpoles or metamorphosed forms) of *Bufo americanus, H. versicolor, R. clamitans* and *R. sylvatica.* All tests were conducted with simultaneous irradiation with white light to facilitate photoreactivation (see Grant and Licht [1995] for details of test design). Irradiation of *R. sylvatica* embryos at 20°C for 30 min. (dose 1.7 J/cm^2) twice during embryonic development was lethal, but most embryos survived when irradiated twice for 15 min. (dose 0.85 J/cm^2). In comparison to irradiation at 20°C, a higher proportion of embryos irradiated at 12°C were abnormal, hatching with curvature of the spine and tail kinks (Fig. 1). Tadpoles of *B. americanus, H. versicolor,* and *R. sylvatica* all died after exposure to 0.113 J/cm^2 for 2 min three times weekly throughout their larval life, but 30% of treated tadpoles of *R. clamitans* survived this dose. However, these tadpoles had arrested development (at stages 31–33), a thickened epidermal layer, and increased melanin pigmentation; histological examination of treated *R. clamitans* confirmed increased

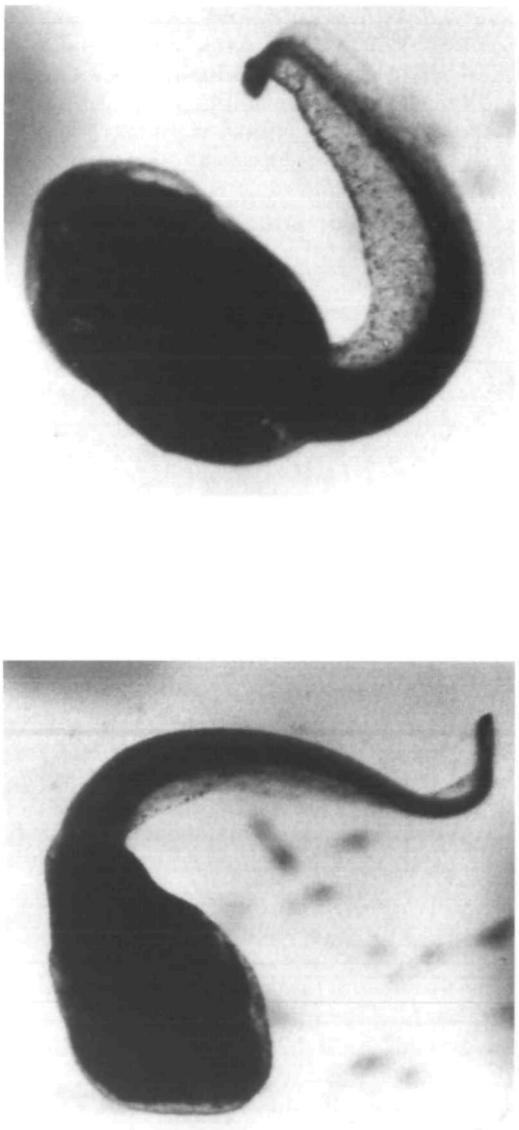

FIG. 1. Abnormal hatchling *Rana sylvatica* (10mm) after exposure to artificially high intensity UVB at 12°C.

melanocytes in the epidermis (personal observation). Metamorphosed *R. clamitans* and *R. sylvatica* died after exposure for 2 min at 0.113 J/cm^2 three times weekly for two weeks, but 30% of treated *B. americanus* survived. Older *B. americanus* survived longer than younger individuals presumably because of thickened epidermis (Grant and Licht, 1995).

All above tests were performed at artificially high intensity levels. Measurements of UVB in Toronto, Ontario, Canada under clear, sunny skies in July 1991, indicated 0.961 J/cm^2 over a four hour period (1000 to 1400 EST using a sensor with spectral peak at 310 nm). This fluence was used for more ecologically relevant testing (Grant and Licht, 1995). When embryos, tadpoles, and newly metamorphosed forms of the above species were irradiated for 4 hr at the simulated outdoor intensity, there were no adverse effects on any stage; neither the survivorship, developmental period, duration of metamorphic climax nor mass at metamorphosis of tadpoles was affected.

The absorption of UVB by jelly surrounding the eggs was confirmed and quantified by Grant and Licht (1995). Segments of jelly (5–6 mm^2 and 3 mm thick) of *B. americanus, R. aurora* and *R. sylvatica* absorbed 6–14% of the UVB. The effectiveness of increasing the thickness of jelly was evident by the finding that entire, clear, masses (devoid of ova) of the salamander *Ambystoma maculatum,* reduced UVB transmission by 61–77%.

Field studies

The diminishing ozone layer and consequent increased UVB recorded over the past decade is the underlying reason for suspecting that UVB may play a part in the apparent decline in amphibians in otherwise undisturbed areas. With the exception of Grant and Licht (1995), most of the above described studies were done with artificially high intensity UVB beyond even predicted levels. Field studies under naturally occurring UVB would be the most relevant approach to test the hypothesis. Because eggs are deposited by many species in oviposition sites fully exposed to sunlight, and because embryos are defenseless and unable to move, the egg stage is suspected to be the one most vulnerable to the effects of UVB.

Blaustein *et al.* (1994) conducted a field study in Oregon (1200–2000 m elevation) to test the effects of UVB on the embryos of several anuran species. They exposed eggs kept in enclosures in natural oviposition sites to several treatments—open sunlight, sunlight filtered through cellulose ac-

etate which allowed 80% UVB transmission, and through Mylar which blocked UVB. They reported that the hatching success of *Hyla regilla* was not affected by UVB, but that of *Bufo boreas* and *Rana cascadae* was decreased after UVB exposure. As well, in a second study (Blaustein *et al.*, 1995*a*), the salamander *Ambystoma gracile* showed increased embryonic mortality after UVB exposure. In laboratory experiments, they found that *H. regilla* eggs had more photolyase, a UVB repair enzyme, compared to the other species and they attributed the results of their field studies to the differential sensitivity to UVB afforded by different photolyase levels. They hypothesized that the reported declines of western North American anurans like *B. boreas* and *R. cascadae* (Fellers and Drost, 1993) may be a result of the apparent differential UVB sensitivity. Their hypothesis of a direct link between natural UVB intensities, photolyase concentration in eggs, egg survivorship in the field, and amphibian decline was questioned (Grant and Licht, 1995; Licht, 1995, 1996; but see Blaustein *et al.*, 1995*b*).

Other attempts to conduct field studies have been completed but not yet published at this time. In three such instances, the experimental design was also to expose eggs to natural levels of sunlight with or without UVB. *Hyla regilla* and *R. pretiosa* were tested (K. Ovaska, personal communication) in British Columbia, Canada at 90 m elevation, as was *B. boreas* in Oregon at 3500 m (S. Corn, personal communication), and *B. americanus, R. catesbeiana, R. clamitans,* and *R. sylvatica* at 100 m in Apsley, Ontario, Canada (M. Berrill, personal communication). In none of these studies was the mortality of embryos exposed to UVB under natural sunlight significantly greater than embryos developing in sunlight with UVB removed by Mylar filters. Moreover, in the British Columbia study, embryos were raised under lamps which enhanced UVB intensities by 30% and in the Apsley study by 50%. In British Columbia, when compared to *H. regilla,* embryos of *R. pretiosa* incurred higher mortality, but in Ontario, the embryos of species studied showed no effects even under the enhanced treatment. The disparity of findings in these field studies with that of Blaustein *et al.* (1994) indicates the importance of continuing field experimentation.

Synergistic studies

The possibility that UVB and other agents in combination may be responsible for amphibian decline has been explored. Kiesecker and Blaustein (1995) reported that a synergistic effect between UVB and a pathogenic fungus, *Saprolegnia,* increased mortality of embryos beyond that from either factor alone. Long *et al.* (1995) found that neither enhanced UVB (9507 eff J/m^2/day) nor pH 4.5 had a significant effect on the survival of *Rana pipiens* eggs, but in a synergistic fashion, the combined UVB and acidity reduced embryonic survival.

Natural defenses against UVB damage

Exposure to UVA and/or visible light (>400 nm) provides the necessary background energy for photolyase, and presumably the new photorepair enzyme (Todo *et al.*, 1993), to repair pyrimidine dimers in damaged DNA (Jagger, 1985). This photorecovery has been documented in several studies with amphibians (Blum and Matthews, 1952; Zimskind and Schisgall, 1955; Worrest and Kimeldorf, 1976) and such a repair mechanism is likely a shared feature in organisms exposed to sunlight at vulnerable stages (Blum *et al.*, 1949). The finding that amphibian eggs have photolyase and that species differ in amounts (Blaustein *et al.*, 1994) may account for the differential sensitivity of some embryos to UVB.

The color of amphibian eggs ranges from cream to nearly black, with melanin pigment typically found in the dorsal hemisphere, or animal pole, of the eggs (Duellman and Trueb, 1986). A clear correlation exists between the oviposition site and exposure to sunlight and egg pigmentation, with eggs hidden from sun lighter in color than those fully exposed (Heyer, 1969). Beudt (1930) showed that the dark eggs of *R. temporaria* were less affected by UV than the lighter pigmented eggs of *Rana esculenta* and noted that melanin was very effective in reducing even intense radiation. Indeed, from a phylogenetic perspective, melanin is considered the most effective

pigment in protecting organisms from UVB damage (Kollias *et al.*, 1991).

A number of jelly capsules surround the ova in amphibians (Salthe, 1963), and the jelly is effective in reducing transmission of UVB through the egg mass as discussed above. Jelly can protect the ova and the thicker the jelly the greater the protection (Beudt, 1930; Gurdon, 1960; Grant and Licht, 1995). The size and shape of the jelly mass will play an important role in such protection. Species of amphibians, like *R. sylvatica* and *R. pipiens*, that breed in spring when waters are cold, often have thick globular egg masses in contrast to surface film layers such as those found in *R. catesbeiana* and *R. clamitans* that spawn in summer (Wright and Wright, 1949). The melanin-covered animal pole absorbs thermal energy and heat is retained effectively by the jelly. Globular egg masses in cold water tend to be higher in temperature than the surrounding water (Herreid and Kinney, 1967; Hassinger, 1970; Licht, 1971; Howard, 1980), in contrast to surface film masses which do not hold heat and, in fact, may be cooler than surrounding water (Ryan, 1978). The thickness of the jelly acting to retain heat would also act to shield the embryos from UVB more effectively and thus the jelly is thicker and more globular in species exposed to sunlight for longer periods. Because the egg developmental rate is temperature dependent (Moore, 1939; Licht, 1971), the eggs in cooler water will develop more slowly and be exposed to UVB for longer periods than the eggs with thin film in warmer water. Grant and Licht (1995) found more abnormalities in *R. sylvatica* embryos which were exposed to UVB at 12° rather than 20°C, and they suggested that the damage was a response to either more irradiation applied to slower developing eggs or to the inefficiency of the photorepair enzyme at lower temperatures. Duellman and Trueb (1986) also noted differences in the size of capsule relative to ova between species that breed in warm or cold waters with the inner capsule size greater in species breeding in cold water. As we hypothesize here for the overall jelly size and shape, the large intracapsular size would afford more UV protection.

One might argue that the species like *R. catesbeiana* and *R. clamitans* that spawn in fully exposed sites in midsummer should have thicker jelly rather than thin surface films. Because they are exposed to higher temperatures, however, and hatch within a few days, they are only subjected to UVB for a brief period compared to cold water breeders. Thus, pressure from overheating appears to have been a greater threat to survival than UVB exposure and a primary factor of selective forces altering the dimensions of jelly surrounding the ova.

Abiotic factors

The factors that affect the amount of UVB reaching the earth's surface were presented above, but amphibian eggs subjected to potential damage from increasing UVB are those that are laid in freshwater aquatic sites exposed to sunlight. Water depth and color are effective barriers to UVB transmission (Smith and Baker, 1979; Scully and Lean, 1995). In an ongoing study of *Ambystoma tigrinum* and *Bufo boreas* from high altitude lakes in Colorado, E. Little (personal communication), has found that humic acids absorb UVB and reduce transmission to embryos. In freshwater (as well as marine), the amount of dissolved organic carbon (DOC) is extremely effective in attenuating UVB in lakes and ponds. For example, in Ontario, in one lake with DOC of 0.5 mg C/liter, the absorption coefficient of UVB was 0.03, whereas in another lake with 7.8 mg C/liter, the UVB absorption coefficient was 23.26 (Scully and Lean, 1995). Coupled with the DOC is the depth factor. In clear tropical marine water, UV (300–400 nm) was attenuated by nearly 33% within the first 2.5 cm (Fleischmann, 1989). In a relatively clear freshwater lake (0.5 mg C/liter) in Ontario, only 34% of surface UVB reached 20 cm, and less than 1% reached the same depth in a lake with a higher DOC of 7.8 mg C/liter (Scully and Lean, 1995). Berrill (personal communication) found that in Ontario, in June, UVB at the water's surface was 0.38% of total incident radiation, but this amount was reduced to 0.1% at only 5 cm below the surface.

Some species of anurans, especially bufonids, lay eggs in shallow water inter-

spersed in vegetation and wound amidst substrate debris (Wright and Wright, 1949). Such debris on the jelly coats of ova can effectively reduce UV penetration to the eggs (Gurdon, 1960; personal observation).

Larval and metamorphosed forms

Most of the physical factors such as water depth and DOC cited as protection for eggs will likewise be effective for larval amphibians. Added to abiotic environmental features is the ability of larvae and metamorphosed forms to move into and out of conditions with fully exposed solar radiation. Thermal regulation is used by aquatic and terrestrial stages (Brattstrom, 1963; Lucas and Reynolds, 1967; Lillywhite, 1970) and the maintenance of optimal temperatures will influence exposure time to UV. Carey (1978) examined the role of skin color and color change in toads with respect to reflectance and absorption of solar radiation and body temperature control. Indeed, a neglected field of investigation is the relationship between heating rate and the potential damage from UVB. In addition, the question of whether or not amphibians are visually sensitive to UV is open (Hailman and Jager, 1976; Jacobs, 1992), and we have preliminary evidence that metamorphosed anurans can preferentially avoid high intensity UVA in discrimination tests (personal observation). Finally, as well as potential behavioral avoidance and/or regulation of exposure to solar radiation, the skin color and glandular secretions, like mucus and external wax (Wygoda, 1988), will protect deeper tissues.

Conclusions

Combined with the mitigating abiotic and biotic factors, current levels of UVB do not appear high enough to support the hypothesis of UV alone as a causative factor of declining amphibians. Nevertheless, given the potential biological damage from increasing UVB in the next decade (Kerr and McElroy, 1993), it is imperative that studies should continue to resolve this important issue. High altitude and/or tropical species, especially those that breed in full sunlight in cool waters of mountain lakes are those that need investigation. As well, adult amphibians which are diurnal at high altitudes may be at increased risk. A survey of interspecific (possibly even intraspecific) levels of photolyase and other protective enzymes, coupled with sensitivity to UVB in all life history stages, would be valuable in predicting which species might be more vulnerable to UVB damage. Laboratory experimentation is important to establish limits of sensitivity between species, but the goal of ecological relevance requires field studies under natural sunlight. While difficult to conduct, such field studies at varying altitudes and latitudes are the ideals to test the hypothesis of the dangers of UVB to amphibian fauna.

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