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Amphibian defenses against ultraviolet-B radiation

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SUMMARY As part of an overall decline in biodiversity, amphibian populations throughout the world are disappearing. There are a number of potential causes for these declines, including those related to environmental changes such as increasing ultraviolet-B (UV-B) radiation due to stratospheric ozone depletion. UV-B radiation can kill amphibian embryos or can cause sublethal effects that can harm amphibians in later life stages. However, amphibians have defenses against UV-B damage that can limit damage or repair it after exposure to UV-B radiation. These include behavioral, physiological, and molecular defenses. These defenses differ interspecifically, with some species more able to cope with exposure to UV-B than others. Unfortunately, the defense mechanisms of many species may not be effective against increasing persistent levels of UV-B radiation that have only been present for the past several decades due to human-induced environmental damage. Moreover, we predict that persistent UV-B–induced mortality and sublethal damage in species without adequate defenses could lead to changes in community structure. In this article we review the effects of UV-B radiation on amphibians and the defenses they use to avoid solar radiation and make some predictions regarding community structure in light of interspecific differences in UV-B tolerance.

INTRODUCTION

Large losses in biodiversity are being documented around the world in almost all classes of plants and animals (Lawton and May 1995). Though the exact number of species being lost is unknown, it has been suggested that the rate of extinction is greater than any known in the last 100,000 years (Wilson 1992). As part of this "biodiversity crisis," populations of many amphibians are in decline and some species have gone extinct (reviewed in Alford and Richards 1999; Houlahan et al. 2000; Blaustein and Kiesecker 2002). In at least some cases, amphibian losses appear to be more severe than losses in other taxa (Pounds et al. 1997). Moreover, declines in amphibian populations are prominent because many of them are occurring in areas that remain relatively undisturbed by humans, such as national parks, conservation areas, and rural areas some distance from urban centers.

There is concern about amphibian population declines in part because amphibians are considered by many biologists to be excellent "bioindicators" of environmental change and contamination (Blaustein 1994; Blaustein and Wake 1995). They have permeable exposed skin (not covered by scales, hair, or feathers) and eggs (not covered by shells) that may readily absorb substances from the environment. The complex life cycles of many species potentially exposes them to both aquatic and terrestrial environmental changes. Moreover, these attributes and the fact that amphibians are ectotherms make them especially sensitive to changes in temperature, precipitation, and increases in ultraviolet (UV) radiation.

Because amphibian population declines are a worldwide phenomenon, global changes in the environment are being investigated in addition to localized phenomena such as habitat destruction, introduced species, disease, and chemical pollution as contributing to the declines (Alford and Richards 1999; Blaustein and Kiesecker 2002). One of these global changes, increasing UV radiation due primarily to stratospheric ozone depletion, is considered to be one important factor involved in the population declines of some amphibian species (Blaustein et al. 2001a; Blaustein and Kiesecker 2002).

Here we briefly review the impacts of UV radiation on amphibians, discuss some of the defenses amphibians may use to limit their exposure to UV radiation, and review some of the mechanisms amphibians use to repair UV-induced damage after exposure.

For a more thorough understanding of ecological problems, such as amphibian population declines, it is becoming increasingly clear that a multidisciplinary approach is essential. For example, factors affecting amphibians during development can eventually affect whole populations. Here we show how developmental biology, molecular genetics, and ecological processes coalesce and are a key to fully understanding important ecological issues.

HISTORICAL SIGNIFICANCE OF UV RADIATION TO AMPHIBIANS

At the terrestrial surface, UV-B (280–315 nm) radiation is extremely important biologically. Critical biomolecules absorb light of higher wavelength (UV-A; 315–400 nm) less efficiently, and stratospheric ozone absorbs most light of lower wavelength (UV-C; 200–280 nm) (Cockell and Blaustein 2001). UV-B radiation can cause mutations and cell death. At the individual level, UV-B radiation can slow growth rates, impair the immune system, and induce various types of sublethal damage (Tevini 1993).

Over evolutionary time, UV radiation has been a ubiquitous stressor on living organisms (Cockell 2001). Natural events such as impact from comets and asteroids, volcanic activity, cosmic events such as supernova explosions, and solar flares can result in large-scale ozone depletion with accompanying relatively rapid transient increases in UV radiation (Cockell and Blaustein 2000; Cockell 2001). The detrimental effects of short-term ozone depletion may only last a few years (Cockell and Blaustein 2000). However, the effects of impacts on stratospheric ozone depends on a number of factors, including the size of the impact (Cockell and Blaustein 2000).

It is likely that exposure to "bursts" of UV radiation historically presents a different situation than what organisms are faced with today. Presently, human-induced production of chlorofluorocarbons and other chemicals continuously deplete stratospheric ozone inducing long-term persistent increases in UV-B radiation at the earth's surface. Furthermore, decreases in stratospheric ozone along with climate warming and lake acidification lead to decreases in dissolved organic carbon concentrations (e.g., Schindler et al. 1996) and result in increasing levels of UV radiation in aquatic systems.

Recent data gathered from remote sensing indicates that levels of UV-B radiation have risen significantly (especially since 1979) in both tropical and temperate regions (Kerr and McElroy 1993; Herman et al. 1996; Middleton et al. 2001). Even though there are shortcomings concerning UV data from remote sensing (discussed in Middeleton et al. 2001; Blaustein and Kiesecker 2002), data showing increasing UV levels are consistent with mounting experimental evidence that UV radiation is harming amphibians in nature (discussed below) and may be contributing to amphibian population declines (Blaustein et al. 1998, 2001a).

Many species of amphibians are subjected to levels of UV-B radiation that could eventually affect whole populations. Moreover, exposure to UV-B radiation may be especially significant for those amphibian species for which selection pressures have resulted in behaviors that expose them to large doses of solar radiation. For example, many amphibians lay eggs in open shallow areas where they receive maximum exposure to sunlight. This exposure can heat egg masses, which induces fast hatching and developmental rates (Stebbins and Cohen 1995). Similarly, the larvae of many amphibian species seek shallow open regions of lakes and ponds, where it is warmest and where they can develop quickly (Hokit and Blaustein 1997; Hoff et al. 1999). Rate of development is especially critical for amphibians living in ephemeral habitats. Species living in temporary habitats must metamorphose before their habitat dries or freezes (Blaustein et al. 2001b). Thus, amphibians are often faced with conflicting selection pressures. Some species must develop quickly enough before their habitat disappears. Therefore, they seek sunlight where exposure to solar radiation enhances development. Yet, evidence from a number of recent studies illustrates that many amphibian species, even those that seek sunlight, are sensitive to solar radiation (e.g., Fite et al. 1998; Belden et al. 2000). If exposed, eggs or larvae may die or individuals may accrue sublethal damage, including slower developmental time (discussed below). For example, frogs that bask in the sun may develop severe retinal damage (Fite et al. 1998).

EFFECTS OF UV-B RADIATION ON AMPHIBIANS

Using field experiments, investigators around the world have shown that ambient UV-B radiation decreases the hatching success of some amphibian species at natural oviposition sites (Blaustein et al. 1994a, 1998, 2001a) (Table 1). For example, in the Pacific Northwest (USA), the hatching success of Cascades frogs (*Rana cascadae*), western toads (*Bufo boreas*), and long-toed (*Ambystoma macrodactylum*) and northwestern (*A. gracile*) salamanders was lower when exposed to ambient UV-B radiation than when eggs were shielded from UV-B (Blaustein et al. 1998). However, the hatching success of spotted (*R. pretiosa* and *R. luteiventris*), red-legged (*R. aurora*), and Pacific tree (*Hyla regilla*) frogs was not significantly different between the UV-shielded and UV-exposed treatments (Blaustein et al. 1998). In California, the hatching success of Pacific treefrogs was not affected by ambient levels of UV-B radiation, but hatching success was lower in California treefrogs (*Hyla cadaverina*) and California newts (*Taricha torosa*) exposed to UV-B (Anzalone et al. 1998). In Europe, the hatching success of common toads (*Bufo bufo)* was lower in UV-B–exposed eggs than in those shielded from UV-B, whereas there was no effect of UV-B on the hatching success of the Natterjack toad (*B. calamita*) (Lizana and Pedraza 1998). The hatching success of moor frogs (*Rana arvalis*) increased when embryos were shielded from UV-B, but there was no effect on hatching success when embryos of common toads (*Bufo bufo*) and common frogs (*Rana temporaria*) were shielded from UV-B (Häkkinen et al. 2001). In Australia, the hatching success of the alpine treefrog (*Litoria verreauxii alpina*) and eastern froglets (*Crineria signifera*) was significantly enhanced when embryos were shielded from UV-B radiation (Broomhall et al. 1999), but hatching success was not affected by UV-B in two species of treefrogs (*Litoria dentata* and *L. peronii*) (van de Mortel and Buttemer 1996).

Table 1. Photolyase activities, egg-laying behavior, and results of field experiments in North American amphibians

Species	Specific Activity of Photolyase 10^{11} CPDs/h/μg[1]	Egg-Laying Behavior/Exposure to Sunlight[2]	Effects of UV-B on Hatching Success in Field Experiments[3]
Frogs and toads			
Ascaphus truei	<0.1	Eggs laid under stones/unexposed	Experiment not conducted
Bufo boreas	1.3	Eggs laid in open often shallow water/high exposure	Hatching success lower in Oregon; no effect in Colorado
Hyla cadaverina	3.5	Eggs laid near surface attached to twigs or other debris/exposed	Hatching success lower
H. regilla	7.5	Eggs laid in open shallow water/high exposure	No effect
H. squirella	5.0	Eggs laid near bottom of ponds/limited exposure	Experiment not conducted
Rana aurora	6.1	Eggs often attached to stiff submerged stem/variable exposure	No effect
R. cascadae	2.4	Eggs laid in open shallow water/high exposure	Hatching success lower
R. luteiventris	6.8	Eggs laid in open shallow water/high exposure	No effect
R. pretiosa	6.6	Eggs laid in open shallow water/high exposure	No effect
Salamanders			
Ambystoma gracile	1.0	Eggs often laid in open water/some exposure	Hatching success lower
A. macrodactylum	0.8	Eggs often laid in open water/some exposure	Hatching success lower
Aneides ferreus	0.4	Eggs laid in cavities in logs or crevices in rocks/not exposed	Experiment not conducted
Batrachoseps wrighti	0.7	Eggs laid in or under logs/not exposed	Experiment not conducted
Plethodon dunni	<0.1	Eggs hidden/not exposed	Experiment not conducted
P. vehiculum	0.5	Eggs hidden/not exposed	Experiment not conducted
Rhyacotriton olympicus	0.3	Eggs laid in cracks in rocks/not exposed	Experiment not conducted
Taricha granulosa	0.2	Eggs hidden/limited exposure	Experiment not conducted

[1] Methods used to calculate photolyase activities are given in Blaustein et al. (1994a) and Hays and Hoffman (1999).

[2] Egg-laying behavior information is from Behler and King (1979), Ashton and Ashton (1988), Nussbaum et al. (1983), and Walls et al. (1992).

[3] See Blaustein et al. (1998) and this text for references regarding the results of field experiments.

CPDs, cyclobutane pyrimidine dimers.

These studies show clear interspecific differences in how the hatching rates of different amphibian species are affected by UV-B radiation even for species that breed at the same sites. These interspecific differences are consistent with what we would expect for any abiotic factor. Even within a given taxon, all species do not respond to every abiotic factor in the same manner.

COMPLEXITY AND SUBLETHAL EFFECTS OF UV-B RADIATION ON AMPHIBIANS

Even though hatching rates of some species may appear unaffected by ambient UV radiation in field experiments, a number of sublethal effects may be accrued by amphibians (Blaustein et al. 2001a). For example, when exposed to UV-B radiation, amphibians may alter their behavior (Nagl and Hofer 1997; Blaustein et al. 2000; Kats et al. 2000), growth and development may be slowed (Belden et al. 2000; Belden and Blaustein 2002a,b; Pahkala et al. 2001; Smith et al. 2000a), or developmental and physiological malformations may be induced (Worrest and Kimeldorf 1976; Blaustein et al. 1997a; Fite et al. 1998; Ankley et al. 2000). Developmental anomalies after exposure to UV-B radiation can occur in all life stages from extremely early embryonic stages (Scharf and Gerhart 1980; Elinson and Pasceri 1989) through adult forms living in nature (Fite et al. 1998). Sublethal effects may become evident in later life stages even in species whose embryos are resistant to UV-B radiation in field experiments. In fact, several recent studies show that the larvae of some amphibian species are more sensitive to UV-B radiation than embryos (Ankley et al. 2002).

Several experimental studies illustrate that early exposure to UV-B radiation causes delayed effects in later stages. For example, UV-B radiation did not influence hatching success of plains leopard frogs (*Rana blairi*), but larval growth and development was slower in individuals exposed to UV-B as embryos (Smith et al. 2000a). Exposure of *Rana temporaria* embryos exposed to UV-B radiation caused no effects on survival rates, frequency of developmental anomalies, or hatching size (Pahkala et al. 2001). However, larvae exposed to UV-B radiation as embryos had an increased frequency of developmental anomalies, metamorphosed later, and were smaller than larvae shielded from UV-B as embryos (Pahkala et al. 2001). Ambient levels of UV-B radiation have no effects on hatching success in red-legged (*R. aurora*) frogs (Blaustein et al. 1998; Ovaska et al. 1997), but larvae exposed to UV-B radiation as embryos were smaller and less developed than those shielded from UV-B radiation (Belden and Blaustein 2002a).

Recent studies have also shown that the effects of UV-B radiation may be subtle and relatively complex. Thus, a number of abiotic and biotic agents may interact synergistically to enhance the detrimental effects of UV-B radiation. For example, recent experimental studies have shown that pH, contaminants, and pathogens may cause enhanced lethal effects in amphibians when combined with UV-B radiation (Blaustein et al. 1997b, 1998; Blaustein and Kiesecker 2002). Moreover, some agents may not be lethal alone, but in the presence of UV-B radiation they may be especially toxic (Long et al. 1995; Hatch and Burton 1998; Hatch and Blaustein 2000).

Environmental changes may also influence the effects of UV-B radiation on amphibians (Blaustein et al. 2001c; Kiesecker et al. 2001). For example, Kiesecker et al. (2001) illustrated the complex interrelationships among global environmental changes and amphibian mortality. They reported that periodic mass mortality of western toad (*Bufo boreas*) embryos in Oregon resulted from a synergism between UV-B radiation and a pathogenic oomycete (*Saprolegnia ferax*). Prior work illustrated that susceptibility to *Saprolegnia* is enhanced when developing eggs are exposed to UV-B radiation (Kiesecker and Blaustein 1995). UV-B exposure was in large part determined by water depth at oviposition sites. Kiesecker et al. (2001) linked El Niño/Southern Oscillation events with decreased winter precipitation in the Oregon Cascade Range. They suggested that less winter snow pack resulted in lower water levels when western toads (*B. boreas*) breed in early spring. Toad embryos developing in shallower water are exposed to higher levels of UV-B radiation, which results in increased mortality from *Saprolegnia* infection. Thus, global events affect UV exposure of local populations.

AMPHIBIAN DEFENSES AGAINST UV-B RADIATION

Exposure to sunlight over evolutionary time has undoubtedly been a strong selection pressure resulting in mechanisms that have helped animals to cope with UV-B radiation (Epel et al. 1999; Cockell 2001). Animals can either prevent UV damage from occurring or repair damage once it occurs (Epel et al. 1999). Thus, amphibians may use molecular, physiological, and behavioral mechanisms to limit their exposure to UV-B radiation or they may repair UV-B–induced damage (Blaustein and Kiesecker 1997; Hofer 2000).

Behavior: limiting exposure to UV-B radiation

Many amphibians limit their exposure to sunlight spatially or temporally. Amphibian species that inhabit dense forests may receive relatively little exposure to UV radiation. Amphibians may live in leaf litter, in crevices, in muddy water, or in deep water, thus limiting their exposure to UV-B radiation. Nocturnal species obviously are not exposed to UV-B radiation.

Egg-laying behavior is particularly important with regard to exposure to UV-B radiation because eggs cannot move from where they were laid. The way in which an amphibian lays its eggs will determine how much UV-B exposure the eggs receive. Thus, eggs laid in clear, open, shallow water are subjected to more UV-B radiation than those laid in deep water or water with high concentrations of dissolved organic carbon that absorb UV (Blaustein et al. 1998; Häder 1997; Xenopoulos and Schindler 2001). Moreover, the eggs of amphibians that are laid in exposed terrestrial surfaces, such as on the tops of leaves or on soil open to sunlight, are exposed to greater doses of UV-B radiation than eggs laid under leaf litter, in cracks, or under rocks.

Although eggs cannot move from where they are laid, larvae or adult stages may be able to seek areas of the habitat where they are not exposed to high levels of UV-B radiation. Thus, the larvae of some amphibian species frequent depths that attenuate UV to levels that may not be harmful (e.g., Belden et al. 2000). In addition, choice experiments have shown that some species may actively select areas with low UV radiation (Nagl and Hofer 1997; van de Mortel and Buttemer 1998; Belden et al. 2000).

Some salamander species wrap their eggs in leaves, which protects them from UV-B radiation (Langhelle et al. 1999; Marco et al. 2001). Recent experimental evidence showed that the eggs of marbled newts (*Triturus marmoratus*) are extremely sensitive to UV-B radiation, but developing embryos wrapped in leaves and protected from UV-B exposure had a much lower mortality rate than exposed embryos not wrapped in leaves (Marco et al. 2001).

Sunscreens and pigmentation

The eggs of amphibians vary in their pigmentation (Duellman and Trueb 1986; Stebbins and Cohen 1995), from extremely light in color to almost black. Duellman and Trueb (1986) reviewed amphibian egg pigmentation and suggested that egg pigmentation characteristics correlated with exposure to solar radiation. In general, eggs that are exposed to sunlight have more pigmentation than those that are not. Amphibians eggs exposed to solar radiation have melanin deposits over the animal hemisphere, whereas those not exposed to sunlight lack melanin. Eggs of amphibians that undergo direct development and that are deposited in areas hidden from sunlight lack pigment. Moreover, eggs deposited in vegetation above water or on the underside of leaves also lack pigment. The eggs of *Phyllomedusa* frog species that wrap their eggs in leaves have unpigmented eggs, but *Phyllomedusa* that lay their eggs in the open have pigmented eggs. Leptodactylid frogs that construct foam nests in open water have pigmented eggs, but those whose foam nests that are concealed have unpigmented eggs. Yet the eggs of some species that are laid in concealed sites may be pigmented (Duellman and Trueb 1986).

Several investigators have suggested that melanin protects eggs from UV radiation (discussed in Duellman and Trueb 1986; Stebbins and Cohen 1995). Jablonski (1998) suggested that melanin production might act as a natural sunscreen and protect developing amphibian embryos from neural tube defects. In accordance with this, Beudt (1930 as cited in Duellman and Trueb 1986) showed that the darker eggs of *Rana temporaria* were more resistant to UV radiation than the lighter eggs of *R. esculenta.* Of course, these results may be explained by interspecific variation in sensitivity.

Pigments in the skin, including melanin, found in larval and adult amphibians may also protect amphibians from UV radiation (Zimskind and Schisgall 1955; Hunsaker and Johnson 1959; Porter 1967; Cockell and Knowland 1999). A recent study by Hofer and Mokri (2000) suggested a role for a specific, nonmelanic, UV-B absorbing substance (UVAS) isolated from the skin of *Rana temporaria* tadpoles in preventing UV-B damage. UVAS in the tadpole skin had maximum absorption in the UV-B range. The concentrations of UVAS found in tadpoles in the field were about the same as those found in tadpoles exposed to UV-B in the laboratory. Hofer and Mokri (2000) suggested that the existence of two powerful sunscreen factors (melanin and UVAS) may explain the high resistance of *R. temporaria* to intense levels of UV-B radiation at high altitudes. Obviously, the existence of sunscreen compounds does not rule out the possibility that other factors are also involved that contribute to the resistance of *R. temporaria* to UV-B radiation (discussed below).

Some amphibians may darken in response to UV-B irradiance (adult *Rana sylvatica*, Roth et al. 1996; embryonic and larval *Hyla versicolor* and *Xenopus laevis*, Zaga et al. 1998; larval *Hyla arborea*, Langhelle et al. 1999). Whether this response effectively protects individuals from UV-B damage is not known. However, a recent study found that in spotted salamander (*Ambystoma maculatum)* embryos, DNA damage occurs in response to UV irradiation despite increased melanin production in the presence of UV-B (Lesser et al. 2001). Moreover, recent laboratory experiments have shown that larval salamanders darken in response to UV-B, but darker individuals do not have higher survivorship than lighter individuals when exposed to UV-B radiation (Belden and Blaustein 2002c). These studies imply that melanin may not provide sufficient protection from UV-B damage in all amphibian species.

Certain microorganisms can synthesize compounds that strongly absorb UV radiation (discussed in Cockell and Blaustein 2001 and references therein). These include mycosporine-like amino acids. Consumers that eat the microorganisms can also obtain these substances. Mycosporine-like amino acids may be extremely important in protecting animals form UV damage. Although it is possible that amphibians may obtain mycosporine-like amino acids, to our knowledge there has been no research examining this.

Our knowledge of the effects of sunscreen pigments in amphibians is rudimentary. Much of the information on the roles of pigments comes from studies of humans. Yet the exact roles of various pigments in human skin that provide protection from UV damage are also still being identified, as are the mechanisms involved in the process (Prota 1992). Although the matter is still open for debate (Wu 1999), in general, mammals with darker skin are less prone to UV-induced skin damage than those with lighter skin (Kollias et al. 1991; Barker et al. 1995).

Egg jelly and heat shock proteins

Amphibians surround their eggs with jelly capsules (Salthe 1963) that may protect some species from UV damage by absorbing damaging wavelengths of light before they reach the embryo (Grant and Licht 1997; Ovaska et al. 1997). The jellies of several species appear to absorb wavelengths in the UV-B range (Grant and Licht 1997; Ovaska et al. 1997). The characteristics of the jelly surrounding developing embryos may play an important role in protection from UV-B radiation. The size, shape, and mode of jelly deposit may influence how much UV-B is absorbed by developing embryos. However, transmission properties of the jelly are probably critical because, as discussed above, field experiments have shown that the embryos of many species are vulnerable to UV-B radiation even with their surrounding jelly capsules intact (Blaustein et al. 1998). Thus, thick jelly with properties that transmit UV-B radiation may be less efficient at protection than thinner jelly coats with poor UV-B transmitting properties. For example, Cascades frogs (*Rana cascadae*) and northwestern salamanders (*Ambystoma gracile*) have relatively thick jelly coats but their embryos show less resistance to UV-B radiation than those of Pacific treefrogs (*Hyla regilla*), whose jelly coat is relatively thin (Blaustein et al. 1994a, 1995). However, the jelly in egg capsules of *H. regilla* were1.5 to 2.0 times more absorbent than those of red-legged (*Rana aurora*) frogs (Ovaska et al. 1997). As described above, the embryos of both of these species are resistant to UV-B radiation.

Heat shock proteins may also play a role in protecting cells from UV-B damage (Feder and Hofmann 1999; Trautinger et al. 1996). Heat shock proteins prevent the denaturation of proteins during exposure to environmental stress, especially temperature stress, but they may also be important in preventing damage from other stressors, such as UV-B radiation. For instance, water stress in plants and oxygen stress in brine shrimp can result in increasing expression of heat shock proteins (Feder and Hofmann 1999). Less is known about their role in preventing UV-B damage, but expression of at least some of these proteins is up-regulated with UV-B exposure in mammals (Trautinger et al. 1996). To our knowledge, no research has been completed on heat shock proteins and the amphibian response to UV-B.

DNA repair

The major damage induced to DNA by UV light is the formation of cyclobutane pyrimidine dimers (CPDs). Formation of these dimers inhibits proper transcription and translation, which can lead to mutation and cell death (Hearst 1995; Sancar and Tang 1993). Amphibian species may be relatively resistant to UV-B if they can remove or repair UV damaged DNA efficiently. One process that might be involved in removing photoproducts is excision repair, which tends to be more common across taxa but can also be energetically costly if more than a single nucleotide requires repair (Sancar and Tang 1993). However, relatively little is known about excision repair in amphibians (Hays et al. 1990). Another important repair process is enzymatic photoreactivation. One enzyme, CPD-photolyase, uses visible light energy (300–500 nm) to remove CPDs (Friedberg et al. 1995). A second related enzyme, [6-4]-photolyase, similarly uses light energy to reverse pyrimidine-[6-4′]-pyrimidone photoproducts ([6-4] photoproducts). Moreover, multiprotein broad specificity excision repair processes can remove CPDs and [6-4] photoproducts. Both mechanisms may be used simultaneously, but excision repair is typically more efficient for [6-4] photoproducts than for CPDs. Thus, CPD-photolyase appears to be the first level of defense against CPDs for many organisms exposed to solar radiation (Pang and Hays 1991; Friedberg et al. 1995).

The relationship between photolyase activities and the sensitivity of embryos to solar radiation was formerly proposed as the "UV Sensitivity Hypothesis," which predicts significant differences among amphibian species with respect to UV repair activities and differential hatching success of embryos exposed to solar radiation, correlation of these differences with expected exposure of eggs to sunlight, and higher repair activities for species whose populations are not declining compared with those whose populations are declining, where exposures to sunlight are similar (Blaustein et al. 1994a).

Research on embryonic amphibians in the Pacific Northwest (USA) has demonstrated a strong correlation between photolyase activity and resistance to UV-B exposure (e.g., Hays et al. 1996; Blaustein et al. 1994a, 1996, 1999). For example, eggs of the most resistant species in field experiments in the Pacific Northwest (e.g., *H. regilla*, *R. aurora*, *R. pretiosa*, and *R. luteiventris*) have higher CPD-photolyase activity than eggs of more susceptible species (e.g., *R. cascadae*, *B. boreas*, *A. macrodactylum*, and *A. gracile*) (Table 1) (Blaustein et al. 1998).

A similar trend exists for three Australian tree frogs, although the correlation is not as strong as in US studies (van de Mortel et al. 1998). Moreover, there was a general correlation between the amount of photolyase activity, exposure of eggs to solar radiation in nature, and, based on limited data, population status. In addition, recent research has demonstrated that photolyase production can be induced in wood frog, *Rana sylvatica*, embryos with exposure to UV-B (Smith et al. 2000b). This highlights the importance of this enzyme in protection from UV-B and implies that the individuals in high UV environments may be capable of up-regulating the synthesis of photolyase.

There is some correlation between photolyase activities and taxon and between photolyase activities and exposure to sunlight. Thus, in general, salamander eggs showed less photolyase activity than anuran eggs (frogs and toads) (Table 1). Most of the salamander species hide their eggs from sunlight or lay them in relatively deep water where there is significant attenuation of UV-B radiation. The anurans with the highest levels of photolyase in eggs tend to lay eggs in shallow water exposed to UV-B radiation. The two anuran species examined that lay their eggs with limited exposure to solar radiation had relatively low levels of photolyase (Blaustein et al. 1994a).

If eggs are damaged by UV-B in field experiments, obviously neither excision repair nor photolyase processes are working effectively. However, if eggs are resistant, it is difficult to determine which combination of excision repair and photolyases is removing damage. Because photoreactivation is probably the most important repair mechanism in amphibians, a parsimonious explanation is that those species with the highest photolyase activities are the most resistant to UV damage.

Even if eggs are laid in the open at high altitudes (where UV levels may be high) and have long developmental periods where they are subjected to prolonged UV-B exposure, they may not be adversely affected by UV-B radiation if they have efficient DNA repair mechanisms. Conversely, species with low photolyase levels may be quite sensitive to UV-B radiation even if they live at very low altitudes or in habitats subjected to relatively low doses of UV-B radiation (Blaustein et al.1995).

Within a species individuals from one population may differ from members of another population in their sensitivity to UV-B radiation. This has recently been shown experimentally in long-toed salamanders (*A. macrodactylum*) (Belden and Blaustein 2002b). The larvae of long-toed salamanders living at low elevations were more sensitive to UV-B radiation than larvae from higher elevation sites. Interpopulational differences in sensitivity to UV-B radiation may be due to differences in the ability to repair DNA damage, pigmentations, jelly coats, or behavioral differences. Unfortunately, little is known, in general, about population variation in resistance to UV-B exposure. Thus, we do not know whether the response to UV-B exposure shows plasticity. Future work examining plasticity in response to UV-B exposure is important.

ECOLOGICAL CONSEQUENCES OF INTERSPECIFIC DIFFERENCES IN UV DEFENSES

UV-B radiation clearly affects a number of amphibian species. The effects depend on the species, life stage, and ecological parameters. The overall loss of amphibians in ecological communities could profoundly affect ecosystems where amphibians are integral components. Through their trophic dynamics within ecological communities, a loss of amphibians could potentially have a severe impact on other organisms, especially their consumers and their prey (Blaustein et al. 1994b; Blaustein and Kiesecker 1997). Interspecific differences in defenses against UV-B radiation could potentially lead to the loss of some species and not others. Moreover, different stages of different species may be affected. For example, larval amphibians influence both the physical and biological parameters of lakes and ponds as they move about and forage (Alford 1999; Hoff et al. 1999). As the larvae of certain species disappear, the larvae of other species may become more common. Changes within the community will depend on the species that are affected by UV-B radiation because different species have different predators and feed on different food items.

For example, in Oregon, Cascades frogs (*Rana cascadae)* and western toads (*Bufo boreas)* whose embryos and larvae are sensitive to UV-B radiation occur sympatrically with Pacific treefrogs (*Hyla regilla*), which are relatively resistant to UV-B radiation at all life stages. If *B. boreas* disappears and *H. regilla* becomes more common, we predict significant changes in habitats where they co-occur. *Bufo boreas* is unpalatable to many vertebrate predators (Peterson and Blaustein 1992), so as *B. boreas* declines in numbers, the larvae of the highly palatable and UV-resistant species, *H. regilla*, may increase in abundance along with its predators. Physical parameters of the habitat could also change as species composition changes. For example, large schools of *B. boreas* (Blaustein 1988) that churn water and move substrate as they forage could be replaced by *H. regilla* tadpoles that form much smaller schools (Blaustein 1988).

CONCLUSIONS

Amphibian populations are declining throughout the world. Numerous factors may be involved in these declines. One contributing factor appears to be increasing UV-B radiation. UV-B radiation can kill developing embryos and can cause sublethal effects in embryos, larvae, and adults. Amphibians have evolved mechanisms that allow them to cope with UV-B radiation. These include behavior, sunscreens, pigments, jelly coats that surround eggs, and mechanisms of repairing UV-induced DNA damage. Some amphibian species are more effective than others at avoiding UV-B damage or repairing it after damage occurs. Nevertheless, the mechanisms used to defend against UV-B radiation in many species may not be effective in light of pervasive, long-term, anthropogenic changes in UV-B levels, a situation that has only become manifested within the last several decades. Therefore, we predict that UV-B radiation will have significant detrimental impact on populations of amphibian species that are especially vulnerable to UV-B radiation. If UV-sensitive species decline in numbers, significant changes within ecological communities are likely to occur.

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