Recent increases in ultraviolet-B (UVB) radiation reaching Earth as a consequence of stratospheric ozone depletion (Kerr and McElroy 1993) may be damaging to organisms. This higher level of UVB has been postulated as a causal factor in the global decline of amphibian populations (e.g., Blaustein et al. 1994), and the hypothesis that amphibians are dying from exposure to UVB has received wide attention in the media, including radio and television science programs, newspapers, popular science magazines (Blaustein 1994, Blaustein and Wake 1995, Blaustein and Johnson 2003), and textbooks (Pough et al. 1999, 2001). It is important to review the characteristics of eggs and egg-laying patterns of amphibians, and of the published work on the potential for damage from UVB, to evaluate the hypothesis so that realistic experimental tests can be devised.

Amphibian defenses against damage from ultraviolet-B radiation

Ultraviolet radiation falls into three categories. The most potent form, ultraviolet-C, does not reach Earth’s surface. Whereas both UVB and ultraviolet-A (UVA) reach Earth’s surface, UVA radiation (with a wavelength of 320 to 400 nanometers [nm]) is not as damaging as UVB. Potential effects of UVA radiation on amphibians have not been well studied, but Grant and Licht (1995) found that neither survival nor development of embryos or tadpoles of three species of frogs were affected after exposure in the laboratory to artificially high doses of UVA radiation. In any case, natural exposure of amphibians to UVA radiation is not an issue, because only the level of UVB radiation is increasing. For this reason, in this article UVA radiation will not be considered further.

UVB wavelengths (280 to 320 nm) contain sufficient energy to disrupt DNA and inflict immunosuppressive, carcinogenic, and mutagenic effects. The physics and biological effects of ultraviolet radiation on cells, organisms, and specifically amphibians can be found in reviews by Jagger (1985) and Licht and Grant (1997). Amphibian species presumed to be at risk from increased UVB radiation would be those that spawn in ponds or lakes and deposit eggs in aquatic sites fully exposed to natural sunlight. The egg stage is most susceptible to UVB, because eggs and developing embryos deposited in aquatic breeding sites by salamanders, frogs, and toads cannot move out of sunlight as mobile larvae and adult animals can. However, several abiotic and biotic factors can protect eggs and embryos from atmospheric UVB radiation:

- Water color associated with high levels of dissolved organic carbon (DOC) and water depth are very effective in attenuating UVB transmission.
- Melanin is considered the most effective pigment in protecting organisms from damage caused by UVB radiation. Amphibian eggs, which range in color from cream to black, vary in the amount of this pigment, with black eggs being the most UVB resistant.
• Amphibian eggs contain photolyase, an enzyme that activates the photorecovery of damaged DNA when embryos are exposed to UVA radiation or to visible wavelengths concurrent with UVB radiation.

• The ova of aquatic-breeding amphibians are surrounded by a number of capsules containing jelly that absorbs UVB radiation and helps shield the enclosed ova. The jelly coat provides not only direct UVB absorption but also indirect protection by virtue of its sticky texture and its tendency to accumulate a covering of plant or pond debris.

I describe each of these factors and present new data on the efficiency of protective mechanisms along with a review of published work on the potential effects of UVB radiation on amphibian embryos.

Water depth and dissolved organic carbon
Amphibians presumed to be at risk from ambient UVB radiation breed in permanent or temporary bodies of water such as lakes, ponds, and marshes. Dissolved organic carbon in the water, which imparts color, is extremely effective in attenuating UVB absorption (Scully and Lean 1994, Morris et al. 1995), although even clear water attenuates UVB absorption to some degree. In one relatively clear lake in Ontario, Canada, with a low DOC level of 0.5 milligram (mg) per liter, only about 34% of surface UVB radiation reached a depth of 20 centimeters (cm). In a lake with a moderate DOC level of 7.8 mg per liter, less than 1% of UVB radiation reached the same depth (Scully and Lean 1994). In general, in ponds and marshes with high DOC levels, 90% of UVB is absorbed in the top 10 cm of water (Berrill and Lean 1998). In a marsh with a relatively high DOC level (13 mg per liter), the surface level of UVB radiation was attenuated to 55% at a depth of 1.4 cm; at 3.0 cm the UVB level was less than 5% of surface values, and at 5.0 cm the UVB level was 2% (Crump et al. 1999). Diamond and colleagues (2002) studied the DOC content of 26 wetlands in northern Minnesota and Wisconsin and found that in 21 of them, UVB levels would not be high enough to result in amphibian mortality or malformation. In essence, UVB radiation penetrates only about the top 10 cm of ponds or lakes with colored water. However, relatively clear water in high-altitude lakes or ponds will still effectively reduce UVB penetration. In a survey of 136 high-altitude lakes in the Pacific Northwest that were potential amphibian breeding sites, Palen and colleagues (2002) found that 85% had levels of DOC high enough to attenuate transmission of UVB radiation and protect amphibian embryos from damage. In fact, most clear lakes in high altitudes are likely to be used by amphibians for breeding only if the watershed area includes woody debris and emergent vegetation, which will add effective levels of UVB-attenuating organic debris and humic acids to the water (Corn 1998). Several researchers, including Corn (1998), Crump and colleagues (1999), Adams and colleagues (2001), and Starnes and colleagues (2000), have stressed the importance of DOC in attenuating UVB transmission in all but the clearest water.

Color of eggs
Amphibian eggs range in color from cream to black. Melanin pigmentation, which imparts the dark color, is typically found on the dorsal hemisphere. Eggs that are hidden from the sun and laid under debris, among leaves, or in foam nests tend to be lighter in color than those that are fully exposed to sunlight. Eggs of aquatic-breeding salamanders tend to be brown, but the eggs and embryos of most species of frogs and toads that breed in open water are nearly black (Wright and Wright 1949). Melanin is considered the most effective pigment in protecting organisms from damage by UVB radiation (Kollias et al. 1991, Wu 1999). More details on the role of melanin as it relates to amphibian embryo, larva, and adult forms can be found in Duellman and Trueb (1986) and Licht and Grant (1997).

Embryonic ultraviolet-B repair and sensitivity
Amphibian eggs contain photolyase, an enzyme used to remove pyrimidine dimers in damaged DNA (Jagger 1985). Exposure to visible light (wavelength > 400 nm) or UVA radiation concomitant with exposure to UVB radiation will provide energy for photolyase activity. The efficiency and effectiveness of photolyase activity in amphibians and other organisms have been well documented (e.g., Blum and Matthews 1952, Kim and Sancar 1993, van de Mortel et al. 1998, Smith MA et al. 2000). Differential sensitivity of embryos to UVB radiation may, in part, be a consequence of different amounts of photolyase in the eggs (Blaustein et al. 1994).

The exact dose of UVB radiation to which embryos are exposed under natural field conditions varies, depending on the combination of initial atmospheric flux and abiotic and biotic protection. Under such circumstances, lethal levels are hard to ascertain, but exposure of embryos in the laboratory to controlled doses of UVB radiation can provide more precise information on the sensitivity of embryos. Embryos of the wood frog Rana sylvatica are not affected by natural ambient UVB levels (Crump et al. 1999), but, unsurprisingly, they do show increased mortality when exposed to artificially high doses of UVB radiation (Grant and Licht 1995). This previous work with R. sylvatica indicated the UVB level that might be lethal to other species in an artificial laboratory setting.

In 2001, eggs of the anurans Bufo americanus, Hyla versicolor, Pseudacris crucifer, Rana catesbeiana, Rana clamitans, and Rana pipiens were collected from natural breeding sites in southern Ontario. At various stage of embryonic development, the eggs were exposed to artificially high doses of UVB radiation and subsequently observed, following the experimental protocol outlined by Grant and Licht (1995). Eggs laid singly, and eggs removed from full intact masses yet still within jelly coats, were placed in petri dishes filled with 2.5 cm of dechlorinated water at 20 °C (±1 °C). For each species, sets of 20 eggs were exposed only once to fluorescent white light.
Figure 1. Percentage mortality of six species of anuran embryos exposed in the laboratory to artificially high doses of ultraviolet-B (UVB) radiation for 0, 15, 30, or 60 minutes (min). Eggs were in petri dishes filled with 2.5 centimeters of dechlorinated water at 20 °C (± 1 °C). Eggs were exposed to fluorescent white light only (0 min) or to white light concurrent with UVB for 15 min (dose of 468 millijoules [mJ] per square centimeter [cm²]), 30 min (936 mJ per cm²), or 60 min (1872 mJ per cm²). The legend for embryonic developmental stages for all species at the onset of exposure is in the upper left corner of the curves for *Bufo americanus*. 

Four cells
Yolk plug
Tall bud
2 days posthatch
7 days posthatch
light or to white light concurrent with UVB radiation (UVB lamp with spectral peak of 302 nm, irradiance of 0.520 milliwatts per square centimeter [mW per cm$^2$]) for 15, 30, or 60 minutes. This exposure resulted in UVB doses of 468, 936, or 1872 millijoules (mJ) per cm$^2$, respectively. To determine whether sensitivity to UVB radiation changes with embryonic development, eggs collected in the early stages identified by Gosner (1960) were exposed to UVB radiation under the treatment protocol at four stages of development (stage 4, four cells; stage 11, yolk plug; stage 17, tail bud; and stage 23, 2 or 7 days after hatching).

The effects of this intense, artificially high UVB exposure at different stages of development are shown in figure 1. Embryos of all species were exposed when in tail bud, allowing comparisons of interspecies sensitivity at that stage. Mortality of $R. \text{clamitans}$ embryos after 30 minutes of exposure to UVB radiation was 35%; overall, however, 15- and 30-minute exposures produced no significant effect on mortality of embryos of the other species (all $P > 0.05$ by logistic regression analysis). After exposure at the same developmental stage (tail bud) for 60 minutes, mortality varied from 70% in $R. \text{clamitans}$ to 15% in $B. \text{americanus}$ and $H. \text{versicolor}$. Mortality was 40% for $P. \text{crucifer}$, $R. \text{catesbeiana}$, and $R. \text{pipiens}$. Embryos of $B. \text{americanus}$ were most resistant under all treatments.

Embryos of $B. \text{americanus}$, $R. \text{clamitans}$, and $R. \text{pipiens}$ were exposed at early (four-cell) and advanced (yolk plug) prehatching stages. For $R. \text{clamitans}$ and $R. \text{pipiens}$, significantly higher mortality after 60 minutes of UVB exposure is seen with advancing development. For $B. \text{americanus}$, however, mortality is still 15%, the same as at early stages. For all time treatments and all species, mortality for posthatching stages is significantly higher than for embryonic stages. Again, hatchlings of $B. \text{americanus}$ were the most resistant. For four-cell embryos of all species, hatching occurred within about 4 to 6 days after the experiment began. Hatchlings were observed for 7 more days for detrimental effects. Mortality occurred in several ways and was most clearly demonstrated after 60 minutes of exposure. Embryos in early stages either failed to advance in development or, for those that did hatch, were curled and twisted, with bent tails and skin lesions. Some hatchlings showed tight, circular swimming movements; they died within a few days. Those embryos first exposed at the 2- and 7-day posthatching (tadpole) stages died within 24 to 48 hours after exposure. Skin lesions and sloughing were common. No lingering morbid behavior was observed.

Further work is required to determine the lethal level of UVB radiation at different stages of embryonic development and to clarify the effects of development on sensitivity. For instance, larger sample sizes might provide more accurate mortality estimates. Also, embryos were exposed to UVB radiation only once rather than several times over consecutive days, and their mortality rates may reflect this experimental design. Nonetheless, this laboratory exposure study yielded an important finding: For embryos of the six anuran species tested, continuous radiation for 60 minutes (with a UVB peak of 302 nm and a flux of 0.520 mW per cm$^2$), at a level far higher than what would be received under natural ambient conditions, was still below a clear lethal threshold. Only advanced embryos of $R. \text{clamitans}$ showed a mortality rate greater than 50%.

In most previous field studies in which embryos showed significantly increased mortality after exposure to ambient UVB radiation, data on the total doses of UVB radiation actually received by embryos were not provided. However, Kiesecker and colleagues (2001) reported that embryos of the toad $Bufo \text{boreas}$, held at a depth of 10 cm in an Oregon lake for 14 days, showed significant mortality when exposed to a UVB flux of about 0.025 mW per cm$^2$. Mortality was thought to be a result of synergism between UVB radiation and the fungus $Saprolegnia$. No data were given on the changing daily levels of ambient UVB radiation in the atmosphere or on the total dose to which embryos were exposed.

Assuming that embryos were exposed to the maximum UVB flux of 0.025 mW per cm$^2$ per second for 6 hours a day, the daily accumulated dose would be 540 mJ per cm$^2$. This is far below the high dose of 1872 mJ per cm$^2$ administered during 60 minutes in the experiment described above, which showed no clear-cut lethal effects on embryos of six species of anurans, including another species of toad, $B. \text{americanus}$. Full, intense sunshine over 14 days might account for increased mortality in the Oregon study, but this information was not provided. (The problematic use of acetate filters in this study will be discussed below.) It is interesting that Corn (1998) found no effects on $B. \text{boreas}$ in lakes in Colorado, although these embryos were exposed to levels of UVB radiation presumably as high as those in Oregon. Furthermore, after a study of the phenology of breeding in amphibians, Corn and Muths (2002) concluded that rather than synergism between UVB radiation and the fungus $Saprolegnia$, exposure to extreme temperatures was an alternative explanation for the embryonic mortality found in the Oregon study.

To summarize, embryos had high natural resistance to UVB radiation—even to artificially enhanced UVB levels—as a result of melanin, photolyase, and jelly around the eggs. Even without added protection from greater depth and from relatively high DOC levels of water in breeding sites, embryos of six species of anurans were very resistant to UVB radiation.

**Larval stage**

Posthatch and larval amphibians are much more sensitive than embryos to UVB radiation. In the six species studied here, high mortality occurs two days after hatching (figure 1). Other studies also report high larval mortality in response to UVB levels that had no effect on embryos (e.g., Grant and Licht 1995, Crump et al. 1999, Hofer and Mokri 2000). The loss of jelly coat protection and diminishing levels of photolyase might account for high mortality in larvae only 2 days after hatching. However, mechanisms such as melanin pigmentation and skin secretions confer some protection (Hofer and Mokri 2000), and, more important, larvae and adults can move in
and out of full intense sunlight. Whether larval or adult amphibians can detect UVB radiation is not known, but temperature regulation and maintenance of optimal thermal conditions would indirectly facilitate regulation of UVB exposure. Thus, although knowledge of the lethal limits of UVB exposure for larval forms may be of interest, experiments that enclose normally mobile animals and restrict movement are not ecologically relevant.

Nevertheless, a number of studies have been done on the development, growth, and behavior of larval amphibians after UVB exposure. References to a number of these studies are found in Blaustein and Kiesecker (2002). Alterations from normal patterns are taken as evidence of the damaging effects of UVB radiation, although the importance of realistic variables in these studies has not been adequately considered. Tests are done in clear water, negating the role of DOC in attenuating UVB exposure; larvae are not given the otherwise normal opportunity to seek refuge from the UVB irradiation; UVB exposure times and doses are usually consistent throughout experimentation and do not account for natural changes and diminishing UVB levels resulting from clouds and overcast skies. Because of these and other factors, the relevance of these studies to potential UVB damage to larval forms in natural settings is minimized. For example, when held in clear water with no opportunity to seek refuge, R. pipiens tadpoles exposed to UVB radiation may develop malformed limbs. Yet the authors of that report, Ankley and colleagues (2000), caution, “The significance of our results relative to field observations of malformed and (or) declining amphibians is unclear. This is due primarily to uncertainties in the actual dose of UV radiation experienced by developing anurans in the field” (p. 1098).

**Jelly envelopes**

The ova of aquatic-breeding amphibians are covered by jelly coats of various dimensions (Salthe 1963). In some species, eggs with associated capsules are laid singly, whereas in other species, females deposit a larger assemblage of eggs interconnected by copious jelly and held within an egg mass. The jelly is a mucoid substance composed primarily of mucopolysaccharides and mucoproteins secreted by the oviduct (Dumont and Brummett 1985). The jelly surrounding amphibian eggs can absorb UVB radiation (Grant and Licht 1995, Ovaska et al. 1997). To make interspecific comparisons of the absorbance capabilities of jelly, numerous species were surveyed. Studies on 12 species representing two orders and five families of amphibians were conducted during the spring and summer of 2001 and 2002 (table 1). Three egg masses from each of two salamander species, Ambystoma jeffersonianum and Ambystoma maculatum, the toad B. americanus, and...
three species of frogs, R. clamitans, R. pipiens, and R. sylvatica, were collected from natural breeding sites in southern Ontario, as were one egg mass from R. catesbeiana and randomly deposited single eggs of Ambystoma laterale and Ambystoma texanum. Two breeding pairs of the frogs H. versicolor and P. crucifer, collected at breeding sites in Ontario, were brought to the laboratory as well, and eggs were laid in aquaria. Two egg masses of the salamander Ambystoma gracile, collected in July 1992 from lakes near Vancouver, British Columbia, had been stored in 5% formalin. For all species, a summary of egg mass size, jelly characteristics, type and depth of egg-laying sites, and time until hatching under natural field conditions is presented in table 1. In 2001, eggs of A. jeffersonianum were collected on 29 March and those of R. catesbeiana on 11 July, with the eggs of the other nine Ontario species collected between these dates. In British Columbia, A. gracile breeds from February to July, depending on altitude (Licht 1975).

Spectral measurements were made on clear jelly removed from egg masses or covering single eggs of all species. The jelly coat around the eggs of A. maculatum can be clear or opaque (Petranka 1998); the egg jelly that was examined was clear. Jelly around the single eggs of A. laterale and A. texanum was retained after the embryos hatched (the jelly from nonviable embryos was removed). For P. crucifer, the single eggs are too small (1.5 millimeters [mm] in diameter) for jelly coat manipulation, and thus the eggs deposited in aquaria were allowed to develop. After the embryos hatched and emerged, the discarded egg capsules of clear jelly were collected. Jelly of each species was placed in a spectrophotometer, using a 1 cm path quartz cuvette and distilled water background. Absorption scans on three jelly samples were run for each species (except P. crucifer, for which only one sample was scanned). Scans were run from 200 to 700 nm; absorption was measured in absorption units (logarithmic scales, with an absorption unit of 1 equalling 90% absorption). Egg jelly from masses of A. gracile and R. sylvatica that had been stored in a solution of 5% formalin was scanned. Identical scans of fresh and preserved eggs of R. sylvatica indicate no effect of formalin on spectral measurements of jelly.

The absorption spectra of egg jelly for A. gracile and R. clamitans are shown in figure 2. These scans are representative of other species. The jelly envelopes of all species show absorbance capabilities within the UVB range (280 to 320 nm). A comparison of absorbance at 300 nm is revealing. A similar overall scan pattern is seen in the jelly of the salamanders A. gracile and A. maculatum, which has the highest absorbance (99%). The pattern of spectral absorbance is nearly identical for the salamanders, A. jeffersonianum, A. laterale, and A. texanum, with about 90% absorbance at 300 nm. At 300 nm, the absorbance of the jelly of R. pipiens and R. sylvatica is about 90%, and that of H. versicolor and P. crucifer is about 80%. The lowest absorbance at 300 nm is for the jelly of B. americanus (70%), R. clamitans (70%), and R. catesbeiana (40%).

To test the effectiveness of the jelly envelopes in protecting the embryos from UVB damage, eggs without jelly were exposed in the laboratory to intense UVB radiation. Field-collected eggs of R. clamitans and B. americanus in the four-cell stage (Gosner 1960) were used. Within the egg mass of R. clamitans, which forms a flat, filmlike surface, each ovum (1.7 mm) is surrounded by a fertilization envelope, a firm jelly capsule 3.0 mm in diameter, and a loose outer capsule 7.0 mm wide. The egg mass of B. americanus appears as long strings, with each ovum (1.5 mm) enclosed in a 2.0 mm capsule within a tube of jelly 5.0 mm wide. For both species the outer jelly capsule was stripped away from an ovum, which left the R. clamitans ovum enclosed in a 3.0 mm capsule and fertilization envelope and the B. americanus ovum enclosed in a 2.0 mm capsule and fertilization envelope.

For each of the two species, sets of 20 dejellied eggs were subjected to the same test conditions as eggs with jelly, thus allowing an evaluation of the effects of jelly on embryonic survivorship. Embryos of R. clamitans in the four-cell stage with jelly removed under 15-, 30-, and 60-minute treatments showed mortality of 15%, 40%, and 90%, respectively. Controls showed 10% mortality. After exposure for 60 minutes, embryos without jelly had significantly higher mortality (90%) than that of embryos with jelly (35%). For B. americanus, four-cell embryos without jelly had mortality rates of 5%, 25%, and 40% after exposures for 15, 30, and 60 minutes, respectively. All controls survived. Mortality of toad embryos exposed for 60 minutes was significantly higher for those without jelly (40%) than for those with jelly (10%).

The embryos of R. clamitans without full jelly capsules exposed for 60 minutes showed the highest mortality. This finding indicates, at least for R. clamitans, that the lethal threshold was approached and that the jelly itself was in fact effective in protecting embryos from the full intensity of the
administered UVB radiation. For *B. americanus*, mortality increased from 10% in jelly-covered eggs to 40% in dejellied eggs, again demonstrating that jelly is efficient at reducing UVB penetration to embryos and that this species is highly resistant to UVB radiation. The jellies of both *R. clamitans* and *B. americanus* are among the least absorptive of UVB radiation; the jellies of the other species, with equivalent size, would most likely be more effective at reducing UVB penetration. Indeed, in an early laboratory investigation into the effects of ultraviolet radiation on uncleaved eggs, Gurdon (1960) pointed out that variability in duration of the radiation dose necessary to get an effect depended on the amount of jelly surrounding the eggs and stated that “it seems necessary for the u.v. rays to break down the jelly before they can reach the egg nucleus.” Thus, egg jelly absorbance is a crucial part of the embryo’s protection from damage by ultraviolet radiation.

**Breeding pattern and egg mass size and shape**

The characteristics of egg-laying sites, egg masses, and time to hatching for species used in this study (table 1) have been described by others (Wright and Wright 1949, Stebbins 1951, Petranka 1998). The various breeding patterns of these species are much the same as the general pattern of all other aquatic-breeding amphibians in the temperate zones of North America and Europe. The jelly of all species shows high absorbance in the critical UVB range. With the increased number of species surveyed here, a clear picture emerges of the interrelated roles of egg jelly, egg mass size and shape, and egg-laying sites in aquatic breeding amphibians.

Of North American amphibians, *A. gracile* and *A. maculatum* have the largest egg masses and the firmest jelly (Stebbins 1951, Petranka 1998). Masses are deposited in early spring in open, cool water (5 °C) of ponds and lakes fully exposed to sunlight, and embryos do not hatch and emerge from jelly for up to 8 weeks. The high UVB absorbance of jelly in the large egg masses of these two species would be especially relevant as protection against radiation, because below 280 nm (figure 2) are not biologically significant that the jelly of all species examined shows high absorbance to UVB radiation, and spectral absorbance for all species is remarkably similar. A peak occurs at 275 nm, as it does in the egg jelly of *Rana aurora* and *Hyla regilla* (Ovaska et al. 1997), but peaks of absorbance at wavelengths below 280 nm (figure 2) are not biologically relevant as protection against radiation, because these wavelengths do not reach the earth. It is relevant that the jelly of all species examined shows high absorbance in the critical UVB range. With a greater number of species surveyed here, a clear picture emerges of the interrelated roles of egg jelly, egg mass size and shape, and egg-laying sites in aquatic breeding amphibians.

**Table 2. Published studies on possible effects of ambient ultraviolet-B radiation on amphibian embryos.**

<table>
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<tr>
<th>Species</th>
<th>Significant effects</th>
<th>No significant effects</th>
<th>Reference</th>
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<td>Long et al. (1995)</td>
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<td><em>R. pretiosa</em></td>
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<td><em>Taricha torosa</em></td>
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<td><em>Triturus cristatus</em></td>
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<td>Langhelle et al. (1999)</td>
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*Note:* Findings from the studies are for tests on current ambient levels of UVB radiation in the field or in the laboratory.
effective in protecting embryos under such conditions. In direct contrast to the egg mass and spawning pattern of these two species of salamanders is that of the frogs *R. catesbeiana* and *R. clamitans*. These species lay thin, filmlike egg masses on the water surface from late May to July, when water temperatures may be above 20 °C. The temperature tolerance of these frogs’ eggs is very high, and their eggs hatch in 4 to 7 days (table 1; Wright and Wright 1949). Thus, even though the level of ambient UVB radiation is higher when the surface mass breeders spawn, the eggs are exposed to high temperatures and UVB radiation for only a few days. Nevertheless, the jelly still provides some absorbance. Thick jelly and dark, melanin-pigmented eggs absorb heat, and the temperature within such masses can be higher than that of the surrounding water (Licht 1971). Egg masses of species such as *R. pipiens* and *R. sylvatica* are globular, with sufficient jelly thickness to aid in raising the temperature of the mass. In contrast, the thin surface masses of *R. catesbeiana* and *R. clamitans* do not hold heat and may be cooler than the surrounding water (Ryan 1978). Overall, the size and shape of the jelly and the choice of egg-laying sites reflect traits that maximize heat gain within embryonic thermal tolerance limits, while concurrently providing protection from UVB radiation. Thus, for open-water breeders, the longer the developmental time to hatching, the larger and thicker the jelly surrounding the ova. The same thesis holds true for other species. Salamander species such as *A. jeffersonianum* deposit eggs in a somewhat smaller but very firm jelly mass, which effectively absorbs radiation. Single eggs or small, loose clusters of a few eggs laid by salamanders such as *A. laterale* and *A. texanum* and frogs such as *P. crucifera* are typically laid on the bottom of ponds among vegetation and debris. They are not normally exposed to full sun, yet their eggs are protected by melanin pigmentation and jelly that absorb 80% to 90% of damaging UVB wavelengths. Species of toads like *B. americanus* lay eggs at the shallow edges of ponds, and egg strings are often entwined with debris and vegetation. The jelly of *B. americanus* eggs have some UVB absorbance, and the black eggs hatch within days.

**Field tests**

The methodology and conclusions in the first paper that reported mortality in amphibian embryos after exposure to ambient UVB levels (Blaustein et al. 1994)—and a possible link between UVB radiation and global amphibian decline—prompted questions (Roush 1995, Taylor 1995, Licht 1996). Since that initial study, 21 others have been conducted on the effects of current ambient levels of UVB radiation on the embryonic stage (excluding studies on the larval stage). The experimental design of these studies is similar: A subset of embryos is exposed either to full, natural (or simulated) sunlight; to sunlight with UVB radiation removed by a mylar filter; or to sunlight passed through an acetate or similar filter that allows about 80% transmission of UVB radiation (thus acting as a control for filtered sunlight). The eggs are kept in enclosures 5 to 10 cm underwater while they are exposed to these sunlight conditions. Eggs are maintained until hatching, after which the mortality for the subsets of embryos held under different light treatments is compared.

Table 2 presents a list of species that have been studied and indicates whether significant effects of ambient levels of UVB radiation on embryos of those species have been reported. Significant embryo mortality has been reported for 9 species; no effects have been reported in 42 tests on 25 species. For *B. boreas* and *B. bufon*, some studies reported detrimental effects of UVB radiation (Blaustein et al. 1994, Lizana and Pedraza 1998) and others reported no effects (Corn 1998, Langhelle et al. 1999, Hakkinen et al. 2001). High UVB alone had no effect, but a synergism between UVB and low pH increased mortality of embryos of *R. pipiens* (Long et al. 1995). Lesser and colleagues (2001) found that the salamander *A. maculatum* showed significant mortality from UVB radiation in laboratory tests but no effects in field tests. They noted the importance of protection by dissolved organic carbon in natural pond water. In Sweden, *R. temporaria* is not affected by UVB radiation, and populations may differ with respect to synergistic effects of UVB radiation and low pH (Pahkala et al. 2001a, 2001c). In contrast, Pahkala and colleagues (2001b) found no such synergism between UVB radiation and low pH for embryos of *R. arvalis* in central Sweden. Hakkinen and colleagues (2001) reported a slight effect of UVB radiation on *R. arvalis* embryos in Finland after finding a third of the embryos dead in both UVB-exposed and UVB-shielded treatments. Broomhill and colleagues (2000; not listed in table 2) exposed embryos of Australian *Crinia signifera* and *Litoria alpifica* to UVB radiation, but their experiment did not yield unambiguous results on embryonic UVB exposure, because the same previously exposed embryos were continually irradiated after they hatched. Despite this confounding methodology, only the *L. alpifica* hatchlings showed increased mortality.

A review of the studies that found significant detrimental effects is revealing. With the exception of Langhelle and colleagues (1999), the relevance of the abiotic and biotic defenses of eggs spawned in natural conditions has not been given enough consideration. Langhelle and colleagues (1999) reported reduced hatching in the salamander *Triturus cristatus* under experimental treatment, but they stated that “in nature, *T. cristatus* wrap their eggs in macrophyte leaves, which probably serve to protect their eggs from UV-radiation.” A critical problem with the design of experiments on UVB sensitivity is that eggs are placed in enclosures in very shallow water depths, which do not reflect typical oviposition sites for the species tested. Blaustein and colleagues (1995), who investigated the UVB sensitivity of *A. gracile*, stated that eggs “were immersed in 5–10 cm of natural pond water, a depth at which eggs are often laid (Stebbins 1954, A. R. Blaustein et al., personal observations).” Yet in the Stebbins reference cited above, egg-laying sites for *A. gracile* are described as “clusters attached to objects in water to [a] depth of several feet but often in shallows at margins of ponds” (Stebbins 1954). Other references for *A. gracile* egg-laying sites are also informative. Stebbins (1951) states that masses are fastened to sticks “from
Thus, 5 to 10 cm cannot be considered the typical depth at which *A. gracile* eggs are laid.

In another study by Blaustein and colleagues (1997), *A. macrodactylum* eggs were placed in enclosures of open water 7 cm deep. Again, this open, very shallow water cannot be considered a typical egg-laying site. The eggs of *A. macrodactylum* are deposited “on bottom or attached to grass or other vegetation at edges of ponds” (Stebbins 1954) and “in shallow to deep water, either attached to vegetation or under the surface of logs. Eggs may also be placed loosely on the bottom” (Nussbaum et al. 1983, p. 54). In Alberta, Canada, eggs are laid on vegetation, logs, or rocks and “typically deposited in water > 10 cm deep” (Graham and Powell 1999). Most relevant is that in Deschutes County, Oregon, where Blaustein and colleagues (1997) found that *A. macrodactylum* embryos showed UVB-induced mortality, Walls and colleagues (1993) describe egg-laying sites for this species as “adhering their eggs singly, usually in large groups, to the underside of submerged rocks” (S. Walls, personal observation)” (p. 1544). Thus, exposing these eggs to UVB radiation is unrealistic.

Another limitation of these studies and others like them is that very shallow, open water reduces the effectiveness of DOC in attenuating UVB absorption. In only a few centimeters of lake water with moderate levels of DOC, most UVB absorption is attenuated (Scully and Lean 1994, Morris et. al. 1995). Moreover, pond debris and vegetation often cover egg masses laid in natural sites, even in shallow water. For example, *B. boreas* is another species that shows high mortality of embryos when exposed to UVB radiation in enclosures at depths of 5 to 10 cm (Blaustein et al. 1994). *B. boreas* eggs may be laid in shallow water “ordinarily not deeper than 12 inches and often less than 6 inches;” yet the eggs “often become greatly entwined in vegetation” (Stebbins 1951).

Another problem in the design of experiments on UVB exposure is the removal of eggs from masses, which diminishes the jelly’s absorption of UVB radiation. *A. gracile* has egg masses that are among the largest of any North American amphibian. Egg clusters have been measured at 2 to 6 inches by 2 to 3 inches of viscous jelly (Stebbins 1951), and firm, globular masses are 180 to 150 mm in diameter (Nussbaum et al. 1983). Marco (2001) reports that “eggs of this species are surrounded by a thick and firm extracellular jelly matrix,” and Marco and Blaustein (2000) point out that gelatinous envelopes protect eggs from mechanical damage, desiccation, predation, and other damaging agents (although they do not mention UVB radiation as one of these agents). In their tests with UVB exposure and *A. gracile*, Blaustein and colleagues (1995) placed 10 eggs from each of seven different clutches and 5 eggs from another clutch (a total of 75 eggs) into enclosures immersed in 5 to 10 cm of water. They noted that the eggs had their jelly matrix intact, but each subset of 10 eggs removed from normal large, intact, firm clusters was thus exposed to UVB radiation without the full protection of jelly absorption that it would have had in the original mass. Thus, the statement by Blaustein and Belden (2003) that “field experiments have shown that the embryos of many species are vulnerable to UVB radiation even with their surrounding jelly capsules intact” might not be applicable if the entire jelly mass, as naturally laid, were to be left intact. Moreover, for *A. gracile*, a water depth of 5 to 10 cm would not be sufficient to cover most intact egg clusters.

A final problem with the experimental design in studies showing harmful effects of UVB is the use of acetate filters placed over eggs and used as a control. Berrill and Lean (1998) found that such acetate filters used to cover underwater enclosures did not affect embryos but were highly toxic to hatchlings. Indeed, Crump and colleagues (1999) noted the importance of being aware of the toxicity of acetate filters: “This observation casts doubt on previous experiments where cellulose acetate was in contact with water (e.g., see Blaustein et al. 1994, 1995)” (p. 1958). In the study by Kiesecker and colleagues (2001) discussed above, in which synergism between UVB radiation and the fungus *Saprolegnia* was presumed, the finding of significant mortality was based on results with embryos and hatchlings in enclosures with acetate filters.

**Conclusion**

The effects of increasing ambient UVB radiation are likely to be most damaging to amphibians living in high-altitude ponds and lakes with relatively low DOC content. Yet recent surveys (Adams et al. 2001, Palen et al. 2002) of such high-altitude amphibian breeding sites in western North America revealed that most lakes had DOC levels high enough to protect species of amphibians thought to be in decline in the area. In fact, Palen and colleagues (2002) concluded, “Until tested at the landscape scale and related to actual population dynamics, the UV-B explanation for amphibian declines should be reconsidered” (p. 2956). Of considerable significance is a recent study by Vance T. Vredenburg (University of California–Berkeley, personal communication, 2003). Vredenburg studied the potential effects of UVB radiation on three species of anurans, *Bufo canorus*, *H. regilla*, and *Rana muscosa*, living in lakes at altitudes above 3000 meters in the Sierra Nevada mountain range of California. The DOC level in the lakes was so low that 90% to nearly 100% of the ambient UVB radiation penetrated through the top 10 cm of water. Field tests were performed with adequate sample sizes and proper controls for all UVB treatments. There was no significant effect of UVB exposure on embryonic survivorship in any of the three species. The relevance of this study of amphibians living in a locality with relatively high levels of UVB radiation and almost clear water cannot be overstated.

Even with the possibility that UVB may affect embryos of some species living in such high-altitude lakes, the ecological significance of that mortality is questionable. Larval amphibian mortality, primarily from predation, is very high (over 90%; Licht 1974), and therefore the consequences of limited embryonic mortality from UVB exposure cannot be
linked to population declines. Aquatic-breeding amphibians typically lay eggs in water that may vary in depth from only a few centimeters to many centimeters or even meters (Duellman and Trueb 1986). During normal breeding seasons, the eggs remain completely submerged. Occasionally, and infrequently (e.g., with receding pond or lake shorelines resulting from minimal, atypical rainfall) for near-surface breeders, the dorsal surface of egg masses may protrude and be exposed to air. However, as Alford and Richards (1999) point out, loss of some embryos from UVB exposure in near-surface egg masses in relatively clear water may have little impact on populations if even a small number of deeper egg clutches survive. In mathematical analyses of factors that might lead to amphibian population decline, Bick and colleagues (2002) and Vonesh and De la Cruz (2002) found that mortality in the amphibian embryonic stage is not as critical as in post-metamorphic life-history stages.

Amphibian embryos are resistant to doses of UVB radiation far higher than those they would normally receive from current ambient levels. Extant amphibians have evolved over a long time period during which varying levels of UVB radiation were most likely a strong selective force. Their adaptive traits include efficient defense mechanisms such as melanin, photolyase, and egg jelly, and they breed in oviposition sites that attenuate most UVB radiation before it reaches the eggs. This combination of factors yields normal high resistance. Relatively few studies have found damaging effects to amphibian embryos from current levels of UVB radiation, and the few studies that report negative effects from UVB have problematic experimental designs. Nevertheless, the hypothesis that effects of ambient UVB radiation are a causative factor in global amphibian decline, or in amphibian deformities, continues to be widely promulgated (e.g., Blaustein and Johnson 2003), even though there has not been a single documented case of amphibian mortality from exposure to UVB radiation under noninvasive natural conditions. When viewed in the light of day, realistic environmental factors and the biology of amphibians do not support the hypothetical link between ambient UVB radiation and amphibian population declines.

Acknowledgments
I thank Duncan Cameron and Joel Shore for critical comments on the manuscript. Access to the spectrophotometer was provided by Brian Colman, and technical assistance was given by Jeffrey Dason and Richard DeMarchi. Collection of bullfrog eggs was made with able fieldwork from Sara Swanson.

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