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## **VARIABLE BREEDING PHENOLOGY AFFECTS THE EXPOSURE OF AMPHIBIAN EMBRYOS TO ULTRAVIOLET RADIATION and OPTICAL CHARACTERISTICS OF NATURAL WATERS PROTECT AMPHIBIANS FROM UV-B IN THE U.S. PACIFIC NORTHWEST: COMMENT**

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Numerous reports suggest that amphibian populations are declining throughout the world (recently reviewed in Alford and Richards 1999, Blaustein and Kiesecker 2002). Potential causes for these declines include habitat destruction, disease, environmental contaminants, global climate change, introduced exotic species, and increasing levels of ultraviolet-B (UV-B; 280–315 nm) radiation. Because of the global nature

of amphibian population declines, much recent effort has focused on understanding the role of increasing UV-B radiation. UV-B radiation is just one of many factors involved in amphibian population declines and it may interact with a variety of other agents (e.g., Blaustein et al. 1998, Blaustein and Kiesecker 2002). Here, we address assertions in two papers that ambient levels of ultraviolet radiation are not harmful to amphibians. Corn and Muths (2002) hypothesize that developing amphibians are not exposed to harmful levels of UV-B radiation if breeding occurs when UV-B levels are low. Palen et al. (2002) hypothesize that amphibians in the western United States breed and live in water whose attributes (e.g., particulate matter, depth, etc.) render UV-B radiation harmless to them. Although we agree with both these possibilities under certain circumstances, at present there is little evidence to support either hypothesis. Conversely, accumulating evidence suggests that UV-B radiation is harmful to many amphibian species. In many cases, for purposes of thermoregulation, amphibians actually seek warm, sunlit habitats with harmful levels of UV-B radiation (e.g., Hutchison and Dupré 1992 and references therein). Furthermore, the authors of both papers narrowly focus on embryos and ignore the growing literature showing that UV-B damages larvae and adults.

To test the Corn and Muths (2002) hypothesis in the most rigorous fashion: (1) breeding behavior, including egg laying, should be observed directly so that one knows when breeding actually occurs, and (2) some regime, preferably using experiments, should be conducted to examine if UV-B radiation actually does harm the amphibians being studied. In addition, if one is interested in whether the specific UV-B levels at the site harm amphibians, measurements of UV-B levels at the actual breeding sites, on the ground, must be taken. Unfortunately, Corn and Muths (2002) did not follow these procedures.

Rather than directly observing amphibians breeding and laying eggs, Corn and Muths (2002) estimated the breeding activity of a single chorus frog species (*Pseudacris maculata*) in Colorado by relying on calling activity estimated from tape recorders placed at a single breeding site. They state that “based on observations of other populations of *Pseudacris*, we have no reason to suspect that egg deposition is not coincident with calling by males.” This method is questionable for several reasons. Most importantly, calling rates are not consistently related to breeding and egg laying. Many chorus frog species call for extremely prolonged periods before breeding and egg laying occur. For example, in Oregon, Pacific treefrog (*Pseudacris regilla*) males may call for more than a month before they mate

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and lay eggs (Nussbaum et al. 1983; A. R. Blaustein, *personal observations*). It is well documented that even choruses of *P. maculata* in Colorado and Wyoming, the species studied by Corn and Muths (2002), begin calling in March and often persist through August, with egg laying occurring throughout that time (Baxter and Stone 1985, Hammerson 1986). Even at higher elevations, *P. maculata* may call from April through June (Koch and Peterson 1995). Thus, the three-day call saturation index, an estimate of the time of maximum calling effort, calculated via remote tape recordings by Corn and Muths (2002) at one location, may not accurately portray breeding in *P. maculata*. In fact, male calling does not always reflect the presence of females, and eggs may never have been laid at that site. According to Corn and Muths (2002) it was too "difficult" to observe breeding and find eggs at that site. We suggest that they should have (1) intensified their effort to observe breeding directly, (2) used additional sites to increase their sample size beyond one, and (3) used more than one species in their analysis for testing their hypothesis more generally. Throughout their paper, Corn and Muths (2002) state that they focus on exposure of "embryos" to UV-B. Yet they did not observe embryonic development in the field.

We suggest that experiments, preferably in the field, are the most rigorous method to examine if ambient UV-B radiation is harming amphibians (Blaustein et al. 1998, 2001a, Blaustein and Kiesecker 2002). Because Corn and Muths did not conduct experiments, we believe that their conclusion that the species they studied in Colorado is not receiving harmful amounts of UV-B radiation is unsupported. Even short-term exposure at very low levels can kill embryos of some species and cause sublethal damage to embryos of many others. For many species, embryos may not appear to be affected after exposure to UV-B. Nevertheless, after embryonic exposure, sublethal effects appear in later stages (larvae and adults; e.g., Smith et al. 2000, Blaustein et al. 2001a, Pakkala et al. 2001, Blaustein and Kiesecker 2002).

Corn and Muths (2002) confound their analysis by using *remote* satellite data to estimate UV-B levels on the *ground*. Instead of measuring UV-B directly at local sites, they relied on indirect and flawed measures of UV-B via satellite-based total ozone mapping spectrometers (TOMS). Regarding the use of TOMS data, Middleton et al. (2001) state that daily estimates of UV-B exposures "at the surface are much more difficult to determine than total column ozone, and the uncertainties of the estimates are due largely to assumptions made in calculations and the limitations related to a single daily observation (e.g., cloud-cover temporal dynamics are lacking)." Furthermore, the "large size of the satellite pixel (50–200 km across) and the necessary

assumption of a homogeneous atmosphere within that pixel" limit the ability to calculate independent UV-B exposure for specific sites within a grid (Middleton et al. 2001). Limitations of satellite data have been discussed in numerous papers (e.g., Mims 1993, Middleton et al. 2001, Blaustein and Kiesecker 2002). Corn and Muths (2002) admit that satellite "estimates of UV-B should be used cautiously, but are also the only estimates available to our long term observations of *P. maculata*." Unfortunately, satellite estimates do not provide accurate measurements of UV-B on the ground. The authors do not adequately address the numerous problems inherent in TOMS data collected from remote satellites, including a lack of data due to complete failure of the instruments for several years (Corn and Muths 2002). For example, the software running the satellite database has had to be constantly revised due to errors in instrument calibration, and data are often inaccurate. Furthermore, and most critically, the resolution of the satellite-generated data is not adequate to approximate ground level interpretations (McPeters et al. 1996). Satellite data thus cannot take into account local conditions influenced by shading, cloud cover, weather patterns, water depth, and dissolved organic carbon (DOC) that can affect exposure of aquatic organisms. In short, data from TOMS are often gross, inaccurate "estimates" of UV-B radiation, especially at ground level (e.g., MCPeters et al. 1996, Middleton et al. 2001).

Corn and Muths (2002) comparison to a study by Kiesecker et al. (2001) is also problematic because both studies used vastly different methods, were conducted in different regions, and examined different species. Thus, Corn and Muths' (2002) broad generalizations about amphibian exposure to UV-B radiation and breeding phenology based on these comparisons are unsupported. Although it is possible to justify the approaches used by the different groups of investigators, it is the comparison between studies that is questionable. Even if the exact same methods and materials are used, it is difficult to compare the results of studies that are conducted in different systems (Blaustein et al. 1998). Thus, Corn and Muths (2002) used indirect satellite-based measurements of UV-B radiation and tape recordings to "estimate" *P. maculata* breeding behavior in Colorado with no tests of how UV-B affected amphibian embryos. In contrast, Kiesecker et al. (2001) measured both of these factors directly at a specific breeding site for *Bufo boreas*. Corn and Muths' (2002) study was a nonexperimental study. Kiesecker et al. (2001) studied western toads in Oregon, using controlled field experiments to specifically test how UV-B affects hatching success. The susceptibility of *P. maculata* to UV-B radiation (or any other agents) is unknown. In contrast, the susceptibility of western

toads to UV-B has been well studied (e.g., Blaustein et al. 1994, Kiesecker and Blaustein 1995, Kiesecker et al. 2001).

As part of their argument against UV-B harming amphibians, Corn and Muths (2002) suggest that temperature extremes are as plausible an explanation as exposure to UV-B radiation for embryo mortality observed by Kiesecker et al. (2001). In criticism of Kiesecker et al. (2001), Corn and Muths (2002) state, "The experiment was not designed to test the effects of temperature on embryo mortality. Therefore, it is unknown whether the more extreme temperatures were responsible for the higher mortality." This statement illustrates their poor understanding of how field experiments are designed. Kiesecker et al. (2001) employed a randomized block design with replicates to examine how water depth, pathogens, and UV-B radiation affect hatching success. This design, regularly used by field ecologists, allows experimental and control treatments to be conducted side by side, after randomly assigning enclosures to positions at the breeding sites. Kiesecker et al. (2001) reported temperatures. There were no significant differences in mean temperatures among treatments, nor were there any block effects. Since there were UV-exposed and UV-blocked treatments at each depth in this experiment, the treatments were subjected to the *same extremes* in temperature. Moreover, numerous previous experiments examining UV-B effects on amphibian embryos also have failed to show a temperature effect (e.g., Blaustein et al. 1994, 1995, 1996, 1999), but none of these papers were cited by Corn and Muths (2002). The design used by Blaustein et al. (1994) and employed by Kiesecker et al. (2001) to investigate the effects of UV-B radiation on amphibian embryos in the field is discussed favorably in a current statistical textbook (Ramsey and Shafer 2002:684–705), a verification of the validity of the design.

We agree with Corn and Muths (2002) that "interactions among two or more factors are likely important for [amphibian] declines. . . ." This has been our main argument in other papers and reviews (e.g., reviews by Blaustein et al. 2001a, 2003, Blaustein and Kiesecker 2002). Yet most studies that have incorporated multifactorial experimental tests were not cited by Corn and Muths (2002). We agree that breeding phenology and UV-B exposure are interrelated (e.g. Blaustein et al. 1994, 1998). A formal conceptual argument for this interrelationship was published by Merilä et al. (2000), which Corn and Muths (2002) also failed to cite.

Palen et al. (2002) estimated the variation in UV-B transparency for 136 "potential" amphibian breeding sites in Oregon and Washington "to determine the proportion of sites that may experience UV-B irradiance levels shown to produce significant mortality in *B. boreas* at another site in the region." They conclude that

most of the sites that they measured had levels of UV-B radiation that would not harm amphibians. Although we admire their landscape approach, we believe that Palen et al. (2002) do not understand how UV-B damage occurs because (1) they fail to understand that dose (exposure to UV-B over time) is the key variable that harms amphibians, and that (2) the UV-B levels (irradiance) that they report as harmless are actually harmful to amphibians. In fact, their study actually supports some experimental fieldwork showing these harmful effects. Unfortunately, as stated in Corn and Muths (2002), "Determining the actual dose received by aquatic stages of amphibians is complex, and depends not only on radiation incident to the surface, but also on water chemistry, location of egg masses, structure of terrestrial and aquatic vegetation (shading), and other factors." Corn and Muths (2002) correctly state, "No study yet has measured the UV-B dose on amphibians in the field."

Palen et al. (2002) concluded that 85% of the ponds they surveyed did not receive UV-B levels high enough to kill amphibian embryos. This included seven sites to evaluate the relationship between UV-B attenuation and dissolved organic matter (DOM). However, some amphibians would be harmed if exposed for a prolonged period of time to the UV-B levels they report in their Table 1 (Palen et al. 2002) as harmless. One problem comes from misuse of the measurements reported by Kiesecker et al. (2001) that formed the basis of their measurements. Apparent lack of awareness of the literature on UV-B levels that harm amphibians adds to the problems with their paper.

Despite the best attempts of Palen et al. (2002), it is simply not possible to correlate single point measurements of UV-B level with dose, because they based their calculations of UV-B flux on a single measurement of surface irradiance at one lake in Oregon (Kiesecker et al. 2001). The UV-B measurements used by Kiesecker et al. (2001) were taken to show that UV-B levels decrease with depth, and do *not* reflect dosage measures or levels that caused mortality. In fact, Kiesecker et al. (2001) selected days and the time of day with the brightest sun to emphasize the attenuation effect of UV-B in the water column. Thus, the level of UV-B, 26.4  $\mu\text{W}/\text{cm}^2$ , reflecting surface irradiance and used in calculations by Palen et al. (2002), was calculated by Kiesecker et al. (2001) to purposely reflect the highest level recorded at the hour of highest exposure on the sunniest days. If Kiesecker et al. (2001) had taken measurements on three overcast days during the experiment, when UV-B levels were, for example, 10  $\mu\text{W}/\text{cm}^2$ , or at night when measurements were 0  $\mu\text{W}/\text{cm}^2$ , the same levels of embryonic mortality would have been reported. Dosage levels determine damage to amphibians, and taking a single measurement at a

single point in time is insignificant. It would be similar to taking a single measurement of temperature, assuming that temperature never fluctuated and that temperature in the pond remained at that single measured constant level.

Palen et al. (2002) may have been unaware of published ranges of UV-B levels and their effects on amphibians in the Pacific Northwest (see for example, Blaustein et al. 1997, Belden et al. 2000, Belden and Blaustein 2002a) and this further dilutes their argument. Although we want to emphasize that dose is the important parameter, for illustrative purposes, we have to ignore the problems of not continuously measuring UV-B levels and disregarding dose. Doing so shows that Palen et al.'s (2002) use of  $22.4 \mu\text{W}/\text{cm}^2$  extrapolated from Kiesecker et al. (2001) as a level of UV-B that causes mortality for toad embryos is the highest level reported in the literature that is lethal to embryos of this species. Similarly, their extrapolation from Kiesecker et al. (2001) of  $12.7 \mu\text{W}/\text{cm}^2$  as a level that does not affect survival can actually be lethal to toad embryos and embryos and larvae of other species (e.g., Worrest and Kimeldorf 1976, Hays et al. 1996, Blaustein et al. 1997).

Palen et al. (2002) use Kiesecker et al.'s (2001) high level of UV-B radiation taken once, at one pond, regarding a single species, and extrapolate its effects to several species found in the Pacific Northwest (Palen et al. 2002:Table 1). In fact, levels much lower than the derived mean levels of UV-B at 10 cm depth depicted in Table 1 of Palen et al. (2002) are lethal to amphibians they list and to other species of amphibians as well. For example, UV-B levels as low as 3–8 and 9–11  $\mu\text{W}/\text{cm}^2$  are lethal to long-toed salamander (*Ambystoma macrodactylum*) and Cascades frog (*Rana cascadae*) larvae, respectively, as well as to western toad (*Bufo boreas*) embryos (e.g., Belden et al. 2000, Hatch and Blaustein 2000). The mean UV-B levels for ponds inhabited by these species and depicted in Palen et al. (2002) are at or above those ranges. Much lower levels are lethal or cause sublethal damage to these species and other species as well. For example, levels much lower than  $22.4 \mu\text{W}/\text{cm}^2$  cause deformities in toads (*B. boreas*) and alter their behavior (e.g., Worrest and Kimeldorf 1976, Hays et al. 1996, Kats et al. 2000). Exposure to UV-B at  $12\text{--}14 \mu\text{W}/\text{cm}^2$  is lethal to eggs of the California treefrog (*Hyla cadaverina*) and California newt (*Taricha torosa*) embryos (Anzalone et al. 1998; L.B. Kats, unpublished manuscript). Levels as low as  $1.28\text{--}1.55 \mu\text{W}/\text{cm}^2$  cause inhibition of growth and development in larval red-legged frogs (*Rana aurora*) from Oregon (Belden and Blaustein 2002b). We emphasize it is these levels over time that cause damage, not the instantaneous exposure. One reason that red-legged frogs might be affected by such low levels

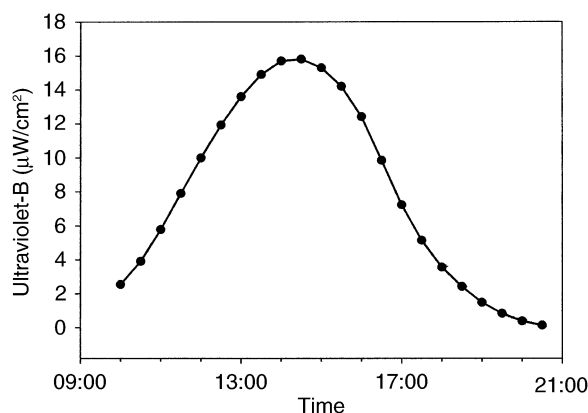


FIG. 1. Fluctuating levels of ultraviolet-B radiation taken on the ground at a stationary position on 17 August 2003 at Corvallis, Oregon (elevation 137 m;  $44^\circ\text{N}$ ), with a Solar Light Company (Philadelphia, Pennsylvania, USA) PMA2100 UV meter.

of exposure is because they develop in very cold water (just above freezing), so that development is slow and they are exposed to low-level UV-B radiation for a prolonged period of time (Belden and Blaustein 2002b). The time component of exposure cannot be ignored.

There are also differences in sensitivity to UV-B radiation in different populations of a single species. For example, valley populations of long-toed salamanders are more susceptible to UV-B radiation than populations from the mountains (e.g., Belden and Blaustein 2002a).

Measurements taken once or twice per pond and in different years (Palen et al. 2002) do not accurately represent amphibian exposure to UV-B radiation. UV-B radiation on the ground fluctuates minute by minute, daily, and seasonally and is influenced by a variety of other parameters (Fig. 1). Both biotic and abiotic factors fluctuate and influence amphibian exposure to UV-B. This includes DOM, incident solar radiation, water levels, and living organisms. For example, Kiesecker et al. (2001) clearly demonstrated the importance of water depth in regulating exposure to UV-B radiation and that water depth can change from year to year. In fact, Palen et al. (2002) claim that in lower water years, DOM is less abundant. Yet variation in water depth was not addressed by Palen et al. (2002), even though they collected their samples over several years.

Indeed, there are many aspects of an amphibian's life history that influence their exposure to UV-B radiation. For example, many species lay their eggs in very shallow water. Often the water is so shallow that the eggs are above the surface open to the air and exposed to high levels of UV-B radiation (Nussbaum et al. 1983, Blaustein et al. 1994). Moreover, as ponds dry, am-



phibians are exposed to higher levels of UV-B. In fact, some ponds dry completely before amphibians can metamorphose and leave the pond. When this occurs, amphibian larvae are stranded with no cover, and are subjected to intense levels of UV-B radiation (see Blaustein et al. 2001b:Fig. 1). Because exposure to UV-B may significantly slow rates of growth and development, amphibians may not be able to leave ephemeral ponds and they may desiccate (Blaustein et al. 2001b, Blaustein and Belden 2003). Every species listed in Table 1 in Palen et al. (2002:Table 1) may lay its eggs in shallow water subject to drying and intense UV-B exposure. For example, in Oregon, and in many other parts of its range, the long-toed salamander (*A. macrodactylum*), a species discussed in Palen et al. (2002) as not receiving harmful doses of UV-B, usually attaches its clutches "to rigid branches or sticks suspended in the water column" (Nussbaum et al. 1983). "When the water level decreases, clutches can remain suspended above the water or can become stranded on the soil" (Marco and Blaustein 1998).

Eggs of many amphibian species are laid in shallow water, and larvae often seek shallow water with thermal gradients that optimize their growth and development and where they are exposed to high levels of UV-B radiation (discussed and quantified in Belden et al. 2000). Many frog species bask in sunlight for prolonged periods of time where they are exposed to high levels of UV-B radiation that can cause significant damage to their eyes and perhaps other portions of their body (Fite et al. 1998; see also Corn and Muths 2002: Plate 1). These are important aspects not addressed by Palen et al. (2002). Even limited exposure to small doses of UV-B radiation can damage amphibians. This depends upon the species and the defense mechanisms that amphibians have to cope with the harmful effects of UV-B exposure (Blaustein and Belden 2003). Thus, an amphibian with relatively efficient molecular defenses against UV-induced DNA damage may be able to withstand long-term high-level UV exposure (high doses). Conversely, species with less efficient defense mechanisms may not be able to cope with even short-term low-level exposure to UV-B (low doses).

Corn and Muths (2002) and Palen et al. (2002) overlooked an increasing amount of data showing that UV-B radiation is harmful to many amphibian species (recent reviews are in Blaustein et al. 2001a, Blaustein and Kiesecker 2002, Blaustein and Belden 2003). Depending upon the species, the life stages that are affected by UV-B exposure may differ. For example, using experiments, numerous investigators from around the world have shown that ambient levels of UV-B radiation reduces hatching success in amphibian species at natural oviposition sites. These include studies in Spain (Lizana and Pedraza 1998, Marco et al. 2001),

Australia (Broomhall et al. 2000), Finland (Häkkinen et al. 2001), and North America (e.g., Anzalone et al. 1998, Blaustein et al. 2001a) among others. Sublethal effects also were overlooked by both Corn and Muths (2002) and Palen et al. (2002). At least 29 frog, toad, and salamander species from North America, Europe, Africa, and Australia accrue sublethal effects after exposure to ambient levels of UV-B radiation (reviewed in Blaustein et al. 2003). Many of these effects are apparent only after the embryonic stage. These include malformations of the body, eyes, and limbs, internal physiological deformities, aberrant behaviors, and decreased growth and development. Moreover, Corn and Muths (2002) and Palen et al. (2002) failed to acknowledge numerous studies showing that UV-B radiation interacts synergistically with environmental contaminants and pathogens (reviewed recently by Blaustein et al. 2001a, 2003; specific examples are Kiesecker and Blaustein 1995, Long et al. 1995, Zaga et al. 1998, Hatch and Blaustein 2000).

Palen et al. (2002) found no amphibians in 32% ( $N = 53$ ) of the ponds they surveyed. They concluded, "the current distribution of amphibian breeding sites is due not to a recent elimination of amphibians from high UV-B areas, but rather a rather long-term adaptation to avoid high UV-B." We disagree with this conclusion for several reasons. Details of their surveys were not provided, but it appears that ponds were only surveyed for one or two days. It is possible that Palen et al. (2002) conducted surveys when amphibians were not active or missed them due to the small effort of surveying. Many amphibians are active for only short time periods seasonally or on a daily basis, especially those in montane habitats. Second, ponds where amphibians were not found may simply be uninhabitable for amphibians and this may have nothing to do with levels of UV-B radiation. Amphibians may have never occupied them. These lakes may not have biotic or abiotic parameters necessary to sustain amphibians or to attract amphibians for breeding. Essential food, shelter, dissolved oxygen, or thermal requirements may be missing. Key predators or competitors may be present. The ponds may be contaminated, too acidic or not acidic enough. None of these site factors were reported by Palen et al. (2002). To examine if these uninhabited ponds are at least habitable, you would add amphibians to the pond and observe if they survive. This obvious experiment was not performed.

Amphibians are faced with conflicting selection pressures (e.g., Blaustein and Belden 2003). Some species must develop quickly before their habitat disappears. Therefore, they seek sunlight where exposure to solar radiation enhances development. Yet evidence from recent studies illustrates that many species, even those that seek sunlight, are harmed by solar radiation. For

example, eggs of Cascades frogs and western toads are laid in open shallow water (Blaustein et al. 1994). Larval Cascades frogs, western toads, and long-toed salamanders, all species discussed in Palen et al. (2002), seek shallow water with the highest temperatures so that they can leave before the pond dries or freezes (e.g., Hokit and Blaustein 1997, O'Hara and Blaustein 1982). Yet all these species are harmed by UV-B radiation.

Selection pressure over evolutionary time for seeking thermal regimes that maximize growth and development were probably important in shaping the behavior of many amphibian species (Hutchison and Dupre' 1992 and references therein). This is especially true for the species we have studied and reported in Palen et al. (2002) (e.g., O'Hara 1981, Hokit and Blaustein 1997). Although UV-B has been a ubiquitous stressor on living organisms since life began (Cockell and Blaustein 2001), increasing levels of UV-B have been occurring due to anthropogenic reasons for <100 years (Cockell and Blaustein 2001 and references therein). Thus, increasing UV-B radiation is a relatively recent selection pressure, and behaviors to limit amphibian exposure to sunlight are unlikely to have overridden strong established selection pressures for seeking warm water and sunlight to enhance their growth. Instead of a "long-term adaptation" (Palen et al. 2002) for avoiding regions with high UV-B levels, there is in fact, a long-term adaptation for seeking out those regions. It is more likely, then, that amphibians seek sunlight, but without effective defense mechanisms against harmful UV-B rays, they die or are damaged when exposed to UV-B radiation (Blaustein and Belden 2003).

There is a large and growing body of literature on the harmful effects of UV-B on amphibians (reviewed in Blaustein et al. 2001a, Blaustein and Kiesecker 2002). Understanding how UV-B affects amphibians is probably greater than for any other factor hypothesized to contribute to amphibian population declines (See Alford and Richards 1999, Blaustein and Kiesecker 2002). This is because there has been a step-by-step progression from early laboratory tests conducted in the 1970s to field experiments conducted in the 1990s on the effects of UV-B on embryos and hatching success (reviewed in Blaustein et al. 1998). The experiments were based on long-term observations in the field (e.g., Lizana and Pedraza 1998, Blaustein and Belden 2003 and references therein). Studies detailing the effects of UV-B on larvae and adults followed. More recent studies have concentrated on sublethal and synergistic effects. Complex dynamics among weather patterns, disease, and UV-B radiation have recently been discussed and illustrated (Pounds et al. 1999, Kiesecker et al. 2001). Our knowledge of the effects of UV-B has

become more sophisticated following the development of models that have incorporated the effects of UV-B on various life history stages. These models illustrate how losses at various stages may affect amphibians at the population level (e.g., Biek et al. 2002, Vonesh and De La Cruz 2002). We are just beginning to understand how specific agents may contribute to the population declines of amphibians. A step-by-step analysis incorporating long-term observations, experiments, and models should be an example for investigating how any specific agent affects the population dynamics of amphibians. Obviously numerous agents contribute to amphibian population declines. Parsimoniously, UV-B radiation is one of many variables affecting the populations of some (but not all) amphibian species.

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