

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

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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

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 affects 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

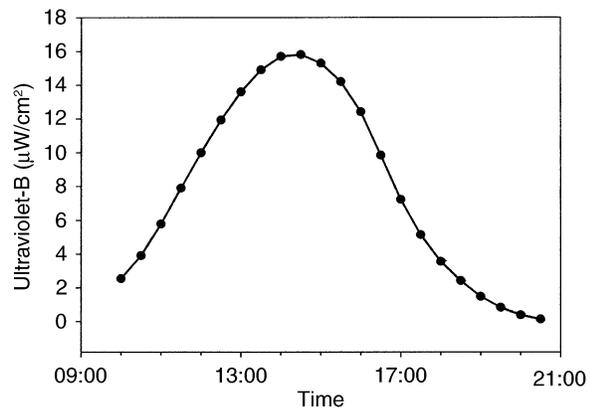


FIG. 1. Fluctuating levels of ultraviolet-B radiation taken on the ground at a stationary position on 17 August 2003 at Corvallis, Oregon (elevation 137 m; 44°N), with a Solar Light Company (Philadelphia, Pennsylvania, USA) PMA2100 UV meter.

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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OPTICAL CHARACTERISTICS OF NATURAL WATERS PROTECT AM- PHIBIANS FROM UV-B IN THE U.S. PACIFIC NORTHWEST: REPLY

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Few ecologists would dispute that exposure to high levels of ultraviolet-B radiation (UV-B) is detrimental to organisms. It is well established that UV-B has been a critical factor shaping the physiology (Blum et al. 1949, Hansson 2000), behavior (Pennington and Emlet 1986, van de Mortel and Buttemer 1998), and distribution (Williamson et al. 2001, Leavitt et al. 2003) of many aquatic species. Recently, increasing UV-B caused by stratospheric ozone depletion has stimulated much research on the UV-B sensitivity of a wide variety of taxa, and has been found to cause direct mortality (Calkins and Thordardottir 1980, reviewed by Siebeck et al. 1994), elevate developmental abnormalities (Ankley et al. 2002), increase susceptibility to disease (Little and Fabacher 1994, Kiesecker and Blaustein

1995), and change the strength of species interactions (Sommaruga 2003). Increasing levels of UV-B have also been invoked as an explanation for the decline of some amphibian species, and support for this hypothesis has been extrapolated from many laboratory experiments and field studies at individual sites that indicate ambient or enhanced levels of UV-B can increase mortality of embryos and larvae (but see Licht 2003). This has been an especially attractive hypothesis for amphibian populations in alpine environments where direct anthropogenic impacts such as habitat modification are limited and ambient levels of UV-B are high (Blaustein and Wake 1990, Blaustein et al. 1994, Alford and Richards 1999). However, for all the attention UV-B has received in the context of declining amphibian populations, there is little evidence linking the physiological sensitivity of individuals to actual population dynamics (Licht 2003).

The mismatch between the context of UV-B experiments and that which is relevant to population dynamics was the primary motivation for an earlier paper (Palen et al. 2002), where we evaluated the spatial variation of UV-B exposure for four Pacific Northwest amphibian species. Previous work by our research group in the Pacific Northwest identified tremendous variation in the penetration of UV-B in the water of alpine ponds used as amphibian breeding sites (Adams et al. 2001). This variation means that amphibians exist within complex landscapes where aquatic breeding sites occur across a wide range of UV-B exposures. It is worth emphasizing that these differences between ponds are not due to variation in the intensity of UV-B reaching the Earth's surface, but rather to differences in the concentration of UV-B attenuating dissolved organic matter (DOM) in the water. This variation in DOM is determined largely by a variety of watershed and wetland characteristics (Malcolm 1990; Brooks et al., *in press*).

Palen et al. (2002) related the variation in optical properties of 136 ponds in the Olympic and Cascade mountain ranges to the optical properties at another site in the region, Lost Lake, Linn County, Oregon, where embryonic mortality has been experimentally linked to UV-B exposure (Kiesecker et al. 2001). We then calculated the proportion of sites that experience either lower or higher UV-B attenuation than Lost Lake

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and found that 85% of sites used for breeding by four amphibian species (*Rana cascadae*, *Ambystoma macrodactylum*, *A. gracile*, *Bufo boreas*) are substantially better protected by DOM from high levels of UV-B than Lost Lake. While we did not directly measure UV-B for every site in the field, we presented evidence from a subset of sites confirming the strong predictive relationship between DOM concentrations and the diffuse attenuation coefficient (K_d) that quantifies the penetration of UV-B through freshwaters (Palen et al. 2002: Fig. 1; also see Scully and Lean 1994, Morris et al. 1995).

In this issue, Blaustein et al. (2004) criticize our approach in a way that misrepresents the original intent and results of our paper. Of primary importance is the false claim that our study suggests UV-B is harmless to amphibians (Blaustein et al. 2004:1747), when in fact our entire analysis was based on the assumption that previous experimental work demonstrating acute sensitivity to UV-B is correct (Kiesecker et al. 2001). Kiesecker and colleagues (2001) conducted an experiment in Lost Lake where they incubated *B. boreas* eggs at three depths and compared the survival in treatments shielded from UV-B to those exposed to ambient conditions. They found a significant negative effect of UV-B exposure at 10 cm water depth, but no difference in survival between treatments at either 50 or 100 cm (Kiesecker et al. 2001), emphasizing the reduction in UV-B effects with increasing depth. Our study builds on this finding by evaluating how commonly the UV-B attenuation conditions documented in Lost Lake are found elsewhere in ponds used by amphibians across montane landscapes of the Pacific Northwest. We found that the UV-B environment at Lost Lake was more intense than at most other sites used by amphibians in our survey (Fig. 1; see also Palen et al. 2002). Even when we eliminate all the sites from our dataset where we did not directly observe amphibians breeding ($n = 53$), as these sites are questioned by Blaustein et al. (2004), our conclusions about the relative UV-B exposure of Lost Lake compared to other breeding sites do not change (Fig. 1B: 84% of sites better protected than Lost Lake). Lost Lake remains among the lakes with the highest transmission of surface UV-B in the Pacific Northwest.

To make this comparison, we made several assumptions about the UV-B exposure of ponds and the distribution of embryos within sites, and in each case we took a liberal approach to favor the conclusion that UV-B exposure is higher in a greater proportion of ponds. First, we assumed that each site in our data set is exposed to the total ambient level of UV-B, ignoring the often substantial effects of shading by local topographic features and vegetation at individual sites. We also assume that all embryos are uniformly distributed

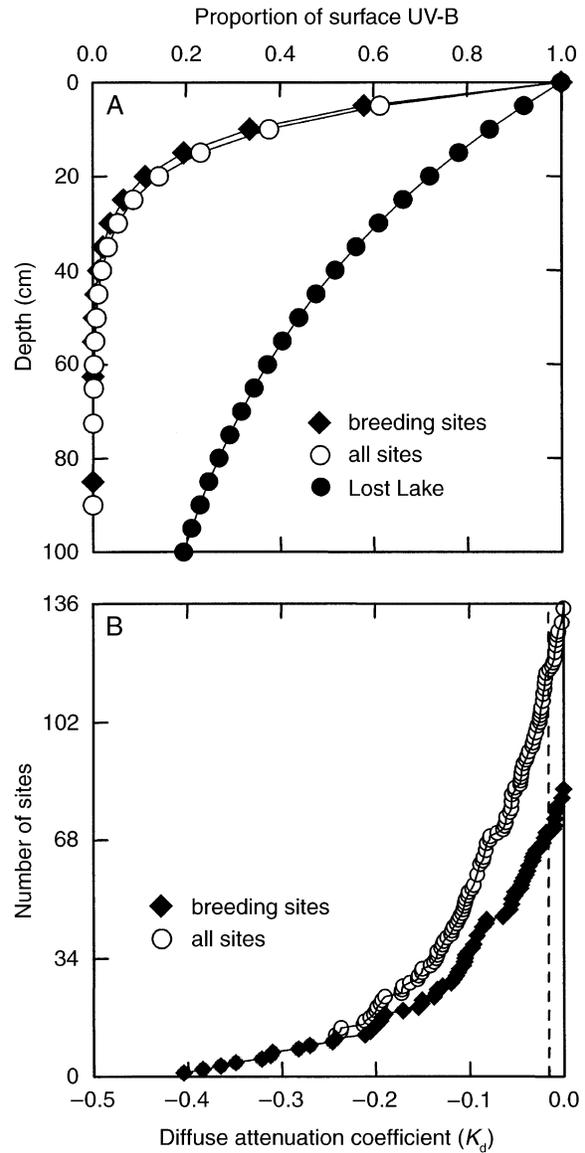


FIG. 1. (A) Average extinction of UV-B radiation with water depth for 136 ponds in the Olympic and Cascade ranges (open circles, average $K_d = -0.09756$, data from Palen et al. [2002]), utilizing only those sites where amphibian breeding was directly observed ($n = 83$, solid diamonds, average $K_d = -0.10897$), and Lost Lake (solid circles, $K_d = -0.01643$, Linn County, Oregon; data from Kiesecker et al. [2001]). (B) Distribution of diffuse attenuation coefficients for all sites (open circles) from Palen et al. (2002) and the subset of sites where breeding was observed (solid diamonds). The dashed vertical line represents the K_d for Lost Lake.

at 10 cm water depth, although at any breeding site embryos occur over a distribution of water depths. There is evidence that at least one of our study species (*A. gracile*) commonly deposits egg masses at depths

exceeding 10 cm (50–100 cm; Licht 1975, Nussbaum et al. 1983), and that another species (*A. macrodactylum*) appears to reduce embryonic exposure to UV-B in low DOM sites by altering its oviposition behavior (W. J. Palen, unpublished data). Finally, we also included sites in our analysis where we did not observe any evidence of breeding by amphibians, recognizing that the patchwork of potential breeding sites may vary through time, such that any one site may be empty in a given year. That each of these assumptions increases the chance of characterizing a pond as having UV-B transmission equal to or greater than Lost Lake only strengthens our conclusion that most of the ponds we sampled are better protected from UV-B than Lost Lake.

While we are criticized for doing so, a fundamental component of our analysis is that we compared the UV-B exposure of each site in our dataset to a focal experimental site (Lost Lake) that has featured prominently in arguments documenting UV-B effects on amphibians (Kiesecker et al. 2001). The importance of this single site to the perception of the role UV-B plays in regulating amphibians is not trivial, as experiments from Lost Lake have been included in >10 papers on the topic, several of which have been widely publicized as evidence supporting the UV-B sensitivity hypothesis for amphibian declines (Blaustein et al. 1994, Kiesecker and Blaustein 1995, Blaustein and Wake 1995, Kiesecker et al. 2001, Pounds 2001). Based on our analysis, we suggest that it is incorrect to interpret the results of exposure experiments conducted in Lost Lake as being broadly representative (Fig. 1; see also Palen et al. 2002). Determining the distribution of UV-B attenuation characteristics for aquatic habitats in the Pacific Northwest clearly requires more sampling, but until more data are available, we must conclude that Lost Lake should be considered a worst-case scenario for the impact of UV-B on amphibians.

Lost Lake is the only site we know of for which there are published UV-B attenuation data linked with a field-based experiment demonstrating a negative effect of UV-B exposure for embryonic survival (Kiesecker et al. 2001). It is the combination of these two pieces of data that allow us to compare the optical environment of Lost Lake to other sites in the region, although Blaustein and colleagues claim that we have misused these data by extrapolating from a single point estimate (Blaustein et al. 2004). They suggest that their intention for collecting UV-B irradiance profiles (Kiesecker et al. 2001) on the “days and the time of day with the brightest sun” was “to emphasize the attenuation effect of UV-B in the water column” (Blaustein et al. 2004:1749). However, the diffuse attenuation coefficient (K_d) does not change as a function of the intensity of surface irradiance, and all of our analyses

involving Lost Lake are based on the K_d calculated from their published data (Kiesecker et al. 2001: Fig. 2b). Thus, if the measured surface irradiance at Lost Lake were in fact $10 \mu\text{W}/\text{cm}^2$ rather than $26.4 \mu\text{W}/\text{cm}^2$ as posited by Blaustein and colleagues (2004), we would have concluded that exactly the same proportion of sites were better protected (e.g., higher DOM concentrations) from levels of UV-B experimentally determined to be harmful at Lost Lake. Our analysis did not depend on identifying a single level of UV-B irradiance or dose that is universally associated with mortality, but rather evaluated the relative clarity of sites based on the measured attenuation of UV-B, negating many of the criticisms made by Blaustein and colleagues (2004).

We are also compelled to address the issue of how organisms respond to UV-B exposure, as we are criticized for not appreciating the mechanisms that generate photodamage in natural systems. Blaustein and colleagues state that our analysis is flawed because we do not acknowledge that UV-B dose is the critical factor for determining the effect of UV-B on organisms. We have already demonstrated that our analysis does not depend on calculating an exact dose of UV-B, but rather depends only on the rate of UV-B attenuation (K_d) in the water column and a relative comparison of K_d among sites. Further, for stationary amphibian embryos where no directed movement is possible, the level of UV-B (irradiance) that reaches a particular depth is directly correlated with the dose received through time. Therefore, for embryos, evaluating the relative clarity of breeding sites while accounting for the depth at which eggs are commonly laid gives a first approximation of the relative UV-B exposure between sites, regardless of whether UV-B damage occurs as a result of peak irradiance or dose through time. The true risk that UV-B poses to aquatic organisms is a combination of atmospheric flux of UV-B, landscape properties of sites, DOM in the water, and the behavior and physiology of individuals, each of which may change seasonally or ontogenetically. While no one study has characterized each of these factors simultaneously, understanding the seasonal variation in DOM loadings to sites and the consequent effect on UV-B penetration is a key area of current research (Brooks et al., *in press*), and a critical component to improving our understanding of UV-B exposure for amphibians.

Blaustein and colleagues correctly state that “We are just beginning to understand how specific agents may contribute to the population declines of amphibians” (Blaustein et al. 2004:1752), but fail to mention that a key factor limiting our ability to evaluate the effects of UV-B exposure for amphibians is the general paucity of quantitative information regarding population status. In montane areas of the Pacific Northwest in particular,

there have been no documented long-term population declines with the exception of the Oregon spotted frog (*Rana pretiosa*) (McAllister et al. 1993, Olson 2001, Pearl and Hayes, *in press*). Field exposure experiments have confirmed that ambient UV-B has little effect on Oregon spotted frog embryos, and is not likely to explain declines in that species (Blaustein et al. 1999). For several other western amphibians where definitive data have been published in support of population declines, field survey and experimental approaches evaluating a variety of alternative hypotheses have concluded that factors other than UV-B (nonnative fish introductions, pesticide drift, and land-use change) are likely to be primary threats to those populations (Corn 1998, Knapp and Matthews 2000, Adams et al. 2001, Davidson et al. 2002). Moreover, population models for two of the focal Northwestern amphibian species (*A. macrodactylum* and *B. boreas*) parameterized with data from UV-B exposure experiments (Blaustein et al. 1994), suggest that even substantial mortality in early life history stages may never impact population sizes or growth rates if survival is density dependent in later stages (Biek et al. 2002, Vonesh and de la Cruz 2002). It is worth highlighting that the study by Vonesh and de la Cruz (2002) is misrepresented by Blaustein and colleagues (2004:1752) as supporting the potential for UV-B induced mortality to affect adult population sizes, which is exactly the opposite of the conclusions drawn by the authors.

Finally, Blaustein and colleagues appear to have misinterpreted DOM as "particulate matter" (Blaustein et al. 2004:1747) and do not understand its influence as the fundamental property controlling the photochemistry of freshwaters. Dissolved organic matter is the key feature of aquatic ecosystems that determines the relative exposure of lakes to UV-B (Scully and Lean 1994, Morris et al. 1995), and represents a broad group of molecular compounds derived primarily from terrestrial vegetation and soils (Malcolm 1990, Leavitt et al. 1997, Pienitz and Vincent 2000). Aromatic forms of DOM (humic and fulvic acids) selectively absorb high-energy wavelengths of radiation (Lewis and Tyburczy 1974, Visser 1984, McKnight et al. 1994), including those that are particularly harmful to living organisms (UV-B, 280–320 nm; Scully and Lean 1994, Morris et al. 1995). Delivery and residence time of DOM in lakes is several times more influential to UV-B in the water column than even the large fluctuations in the amount of UV-B that reach the surface of lakes due to ozone depletion (Schindler et al. 1996, Yan et al. 1996, Williamson and Zagarese 2003). Thus, DOM is the principal factor affecting variation in UV-B levels among mountain ponds and lakes.

Despite their claim to the contrary, we are in agreement with Blaustein and colleagues (Blaustein et al.

2004) about the potential importance of UV-B as an ecological stressor for amphibians as well as for freshwater organisms in general. Considering the immense potential for climate-driven changes in DOM loading to aquatic ecosystems and the subsequent effect on UV-B penetration (Schindler et al. 1996, Williamson and Zagarese 2003), we believe that understanding when and where UV-B is expected to influence populations will be of paramount importance for many ecosystems. While our recent analysis (Palen et al. 2002) does not presume to fill this gap, it contributes to the "step-by-step progression" of research promoted by Blaustein and colleagues (2004), and in the process contributes to our understanding of the extent of UV-B effects on amphibian populations. Additionally, we believe that a promising future area of research will involve large-scale experiments directly manipulating DOM and the UV-B environment of sites to test the importance of UV-B at the spatial and temporal scales relevant for populations and communities, as a vital goal for applied ecology is to conduct experiments at the scales relevant to the real world (Levin 1992, Carpenter 1996).

UV-B exposure experiments conducted at single sites under highly controlled conditions provide a foundation for evaluating the larger scale effects of UV-B, but in the absence of a population or landscape context, their link to population declines is highly uncertain. Our study was the broadest assessment to date of the UV-B transmission properties of amphibian habitats. It suggests that the body of literature from Lost Lake represents an extreme case of high UV-B transmission and should not be considered broadly representative. Other studies suggest that the level of mortality demonstrated in these and other experiments is unlikely to cause population losses (Biek et al. 2002, Vonesh and de la Cruz 2002).

The UV-B hypothesis alone or in concert with other factors should be considered as one of several competing hypotheses (*sensu* Hilborn and Mangel 1997) to explain declines of amphibian populations. Data that relate various environmental stressors to *population dynamics* of amphibians in the field are urgently needed to challenge these competing hypotheses and establish the best explanation for declines in these species at local to global scales.

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VARIABLE BREEDING PHENOLOGY AFFECTS THE EXPOSURE OF AMPHIBIAN EMBRYOS TO ULTRAVIOLET RADIATION: REPLY

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Corn and Muths (2002) described how seasonal and annual variation in estimated flux of ultraviolet-B (UV-B) radiation, combined with year-to-year variation in amphibian breeding phenology, introduces considerable variability in the UV-B exposures to amphibians. The response to our paper by Blaustein et al. (2004) misstates the objectives and conclusions of our study, contains other errors of interpretation, and critiques our study for adopting practices that they themselves use. We are confident that an unbiased assessment will show that the conclusions of Corn and Muths (2002) are valid and robust with respect to montane amphibians, and that the criticisms raised by Blaustein et al. (2004) are either invalid or irrelevant.

Blaustein et al. (2004) assert that “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.” In reality, we stated

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nothing resembling that hypothesis, and Blaustein et al. (2004) read more into our paper than is there. Our study (Corn and Muths 2002) was stimulated, in part, by two papers by Joseph Kiesecker and colleagues. Kiesecker and Blaustein (1995) demonstrated that embryo mortality of *Bufo boreas* and Cascade frogs (*Rana cascadae*) was greater in the presence of a pathogenic oomycete, *Saprolegnia ferax*, and UV-B. Kiesecker et al. (2001) observed that boreal toad (*Bufo boreas*) embryos in the Cascade Range in Oregon developed in shallower water after drier winters, and suffered greater mortality from *S. ferax* in those years. Kiesecker et al. (2001) exposed *B. boreas* embryos to ambient sunlight or UV-B reduced conditions at three depths, and found increased mortality only in the shallowest (10-cm) treatment with ambient UV-B. Because UV-B in water attenuates with depth, they concluded that a synergism between *S. ferax* and UV-B would increase mortality of embryos only in years with shallow water. However, this conclusion fails to account for seasonal variation in UV-B (Merilä et al. 2000). The specific goal of Corn and Muths (2002) was to examine the interaction between variation in UV-B and variation in breeding behavior. Because UV-B radiation increases during the spring (a consequence of the Earth’s orbital mechanics), amphibians breeding earlier in the spring will receive, on average, a lower dose of UV-B than if they breed later. Using our long-term observations of boreal chorus frogs (*Pseudacris maculata*) in the Rocky Mountains of Colorado, we found that breeding occurred earlier in years with lower snow pack. We used satellite-based estimates of UV-B exposure and found a significant positive relationship between UV-B and the timing of breeding activity. Corn and Muths (2002) estimated that, during years of low snow pack, the decrease in exposure to UV-B due to breeding earlier could be comparable in magnitude to the increase in UV-B that Kiesecker et al. (2001) attributed to decreased depth.

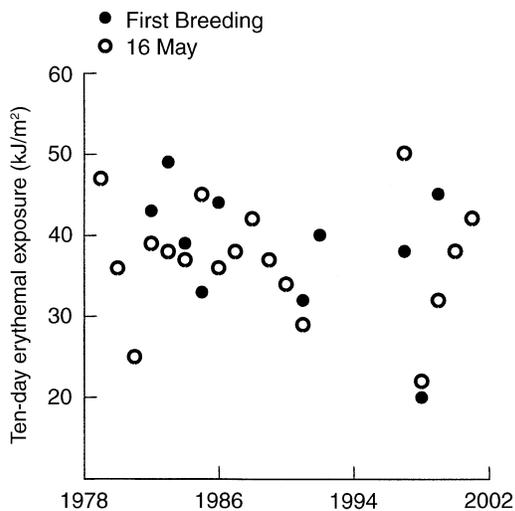


FIG. 1. Estimated 10-day sums of surface UV-B on 16 May, the average date of first breeding by *B. boreas* at Lost Lake in Oregon (open circles) and on the actual dates (solid circles) of first breeding (Blaustein et al. 2001, Corn 2003). The UV-B estimates are for the 1° latitude \times 1.25° longitude pixel centered at 44.5° N, 121.875° W. See Corn and Muths (2002) for methods used to estimate UV-B. There was no relationship between UV-B and year for either set of data.

In their comment on our paper, Blaustein et al. (2004) state that Corn and Muths (2002) conclude, “. . . that the species they studied in Colorado is not receiving harmful amounts of UV-B radiation. . . .” To put it bluntly, there is no such statement or implied claim in our paper. We did not set out to study the effects of UV-B exposure on *P. maculata*, and we agree that well-controlled experiments would be a necessary element of such an effort. Such experiments, however, need to have a strong, “inferential chain to what’s going on in nature” (Joseph Bernardo, in Roush 1995). To hypothesize that increases in UV-B due to ozone depletion have contributed to amphibian declines, it needs to be demonstrated that amphibians have been exposed to increasing UV-B. However, a growing body of evidence suggests that, to date, increasing UV-B dose is a relatively rare occurrence for most species of amphibians. The estimated UV-B exposure of natterjack toads (*Bufo calamita*) in the United Kingdom actually decreased from 1979 to 1994, because warming temperatures have caused breeding to occur earlier, when UV-B flux is lower (Merilä et al. 2000, Cummins 2003). Corn and Muths (2002) found no increase between 1986 and 2001 in the UV-B to which *P. maculata* may be exposed, despite significantly increasing UV-B on the average date of breeding. At Lost Lake in Oregon, the study site used by Kiesecker et al. (2001), there is no evidence that UV-B has increased during the past 25 years (Fig. 1). Middleton et al. (2001) did find increases

in average annual UV-B at 16 sites in Central and South America where amphibian declines have been detected, but the extent to which the species in question are exposed to UV-B is unclear. Several recent studies have investigated the chemistry of waters in which amphibians breed, and all have concluded that the great majority of habitats are protected from harmful levels of UV-B below the surface by high concentrations of dissolved organic matter (DOM) (Adams et al. 2001, Diamond et al. 2002, and *in press*, Palen et al. 2002, Peterson et al. 2002). Field experiments may demonstrate either embryo mortality or sublethal effects on other life stages attributable to ambient UV-B (Blaustein et al. 2004). However, if there is no evidence that ambient UV-B has increased, then the correct conclusion is that these results are part of the natural range of variation in the life history of the species.

Rather than conducting field experiments to test whether *P. maculata* suffers increased mortality under ambient UV-B, Corn and Muths (2002) addressed the justification for such experiments. We used our long-term data on *P. maculata* to estimate the relationships among climatic variation, breeding behavior, and UV-B exposure in a montane amphibian. The phenology of many alpine organisms is strongly influenced by snow pack (Smith and Andersen 1985, Green and Osborne 1998, Inouye et al. 2000). More specifically, *P. maculata* in Colorado as well as the population of *B. boreas* studied by Kiesecker et al. (2001) in Oregon display similar relationships between snow accumulation and timing of breeding (Corn 2003). In fact, we believe that the results for *P. maculata* described by Corn and Muths (2002) should be relevant for many amphibian species breeding in regions with persistent winter snow cover.

Blaustein and Kiesecker (2002) and Blaustein et al. (2003) cited Middleton et al. (2001) as evidence that declining populations of amphibians in Central and South America have been exposed to increasing UV-B. Middleton et al. (2001) used the same satellite-based estimates of surface UV-B as Corn and Muths (2002), yet Blaustein et al. (2004) do not consider these data valid to estimate UV-B exposure in Colorado. Because we used UV-B values associated with specific days, Blaustein et al. (2004) apparently believed that we were reporting the daily UV-B flux estimated from the satellite data. It is true that daily estimates may not accurately represent UV-B flux at the ground. However, we did not use the data in this way. The values we used were not the daily estimates of UV-B, but the sum of the 10 days centered on the date of maximum breeding activity. As stated in Corn and Muths (2002): “Daily TOMS estimates of UV-B can also vary significantly from ground-based measurements, but accuracy is increased when estimates are integrated over several days

(Herman et al. 1999).” Similarly, Middleton et al. (2001) used monthly averages in some of their analyses.

The objection to the use of the satellite-based estimates of UV-B is also related to the misstatement of the objectives and conclusions of our paper. We agree with Blaustein et al. (2004) that satellite data are inadequate to estimate the UV-B dose that would cause damage or mortality. However, this criticism is irrelevant, because Corn and Muths (2002) did not study the effects of UV-B exposure on *P. maculata*. Satellite-based estimates of UV-B flux data are useful for demonstrating seasonal and interannual variation, and this is how we used these data.

Blaustein et al. (2004) cite general accounts of natural history in three field guides to argue that *P. maculata* possesses a prolonged breeding season and that our estimates of maximum breeding activity are invalid. The information in the cited field guides is non-specific and reflects the potential range of breeding activity, over a large elevational gradient (~1500–3600 m) and across years; the interpretation presented by Blaustein et al. (2004) blurs this distinction. For example, Blaustein et al. (2004) state that *P. maculata* in Yellowstone National Park, “. . . may call from April through June (Koch and Peterson 1995).” However, Koch and Peterson (1995:64) clearly state that breeding phenology depends on elevation: “Calling may begin as early as April in some parts of the region and may extend into early July at higher elevations.” *Pseudacris maculata* is an explosive breeder using ephemeral water in the Rocky Mountains (Degenhardt et al. 1996, Hammerson 1999). Phenology information from field guides is not particularly useful for predicting the timing of breeding in a specific wetland during a particular year. Our data were based on 16 years of direct observations of breeding *P. maculata*, including intensive capture–recapture efforts as well as observations of amplexant pairs and egg masses during each year. Our desire to write a succinct Report may have led us to over-economize our description of field methods. We used the index of calling males to characterize breeding activity, because the primary goal of our study at Lily Pond is to study long-term changes in abundance, not to estimate reproductive output. Counting egg masses is difficult at Lily Pond, whether Blaustein et al. (2004) think so or not, but it is clear from our extensive sampling of this population that egg deposition and chorusing by males is coincident. We cannot state with certainty that *maximum* egg deposition coincided with *maximum* chorusing, but we cannot think of a good reason to hypothesize that it did not.

Blaustein et al. (2004) misinterpreted our discussion of the possible role of temperature in mortality of amphibian embryos. Corn and Muths (2002) suggested

extreme temperatures as a possible explanation for episodes of mass mortality of *B. boreas* embryos in Oregon, reported by Blaustein et al. (1994) and Kiesecker et al. (2001). We are aware of no experiments by Blaustein and colleagues that have addressed this hypothesis. Blaustein et al. (2004) are incorrect in stating that the quotation from Corn and Muths (2002) criticized the experiment conducted by Kiesecker et al. (2001). Instead, we were discussing the difference between two sites studied by Corn (1998). Both Corn (1998) and Kiesecker et al. (2001), the former study in Colorado and the latter in Oregon, included a site named Lost Lake, and Blaustein et al. (2004) apparently were confused by this. We continue to assert that the role of temperature in mortality of amphibian embryos is underappreciated and under-studied. We understand the principles of experimental design and appreciate the distinction between controlled and uncontrolled variation. Ours was not an experimental study and we did not criticize the designs of the numerous experiments conducted by Blaustein and colleagues, and we see no justification for doing so in this paper (but see Licht and Grant 1997, Cummins 2002, Licht 2003). Rather, we agree with Merilä et al. (2000) that future experiments should include temperature as a factor.

Corn and Muths (2002) did fail to cite Merilä et al. (2000), and we regret the omission of another study estimating the increase in UV-B flux as the season progresses. However, Merilä et al. (2000) described the interaction between seasonal change in UV-B and latitudinal variation in the timing of breeding by the common frog (*Rana temporaria*) in Sweden, but they did not address the implications of interannual variation in UV-B and breeding phenology at a given location. Lapses in scholarship are regrettably common in science and are becoming more so as the volume of literature increases. For example, Kiesecker et al. (2001) was submitted in September 2000, but they also failed to cite Merilä et al. (2000), although it was published in June 2000.

Blaustein et al. (2004) suggest that Corn and Muths (2002) should have conducted additional research to “increase their sample size beyond one.” This is good advice in general. For example, Pounds (2001) and Blaustein et al. (2003) make broad generalizations about the role of complex interactions of multiple factors in amphibian declines, based on *one* study of *one* species at *one* site (Kiesecker et al. 2001). Our understanding of the causes of amphibian declines will more rapidly advance with additional research by independent groups conducted on more species and in more locations.

Review and criticism of previous studies has its place in science, but the seemingly endless debate over the

role of UV-B in amphibian declines, with its reviews and responses (e.g., Licht and Grant 1997, Blaustein and Kiesecker 2002, Cummins 2002, Kats et al. 2002, Blaustein and Kats 2003, Blaustein et al. 2003, 2004, Licht 2003,) has become redundant. Although Corn and Muths (2002) simply described seasonal and interannual variation in UV-B flux to which a montane amphibian might be exposed, Blaustein et al. (2004) made the issue of UV-B and amphibian declines central to their criticism of our paper, and they repeat arguments made in several other published comments by Blaustein and colleagues. We suggest in this reply that the results of Corn and Muths (2002), Palen et al. (2002), and similar studies indicate that many of the experimental studies cited by Blaustein et al. (2004) have been conducted without adequate justification, because they lack evidence of increasing UV-B. In fact, the results of Kiesecker and Blaustein (1995) and Kiesecker et al. (2001) indicate that mortality of *B. boreas* embryos was not caused by an anthropogenic increase in UV-B, but by other factors that exposed the embryos to naturally occurring levels of UV-B to which they may not be adapted.

Corn and Muths (2002) and this reply should not be construed as denying ozone depletion and increasing UV-B or downplaying the consequences. These are complex phenomena that are difficult to interpret at ecologically relevant local levels, given the tools and data currently available. Although the role of UV-B in causing the amphibian declines that have already been documented is open to question, the interactions between UV-B and other factors, such as climate, pathogens, or contaminants, are likely to be exacerbated if UV-B continues to increase. Future work on amphibians should emphasize these questions.

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