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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 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 Cascades 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.

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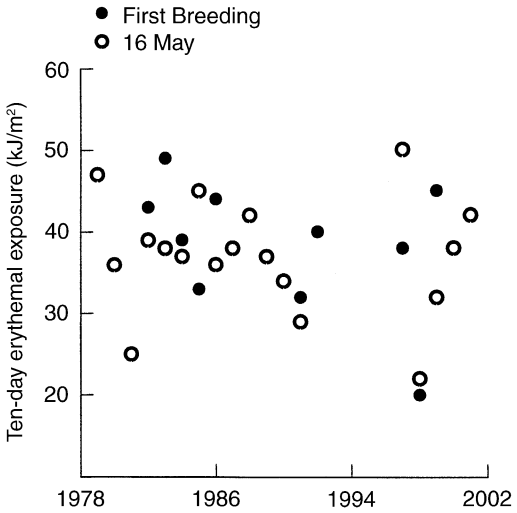

FIG. 1. Estimated 10-day sums of surface UV-B on 16 May, the average date of first breeding by *B. boreas* at Lost Lake in Oregon (open circles) and on the actual dates (solid circles) of first breeding (Blaustein et al. 2001, Corn 2003). The UV-B estimates are for the 1° latitude × 1.25° longitude pixel 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 nonspecific 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 amplectant 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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