AMPHIBIAN DECLINES AND UV RADIATION

In a letter to *BioScience* (45: 307), Lawrence Licht criticized our work on amphibians and ultraviolet (UV) radiation (Blaustein et al. 1994a). Licht's criticisms are unfounded, because he failed to comprehend both our experimental design and statistical analysis. Moreover, unwarranted assumptions and failure to appreciate the power of field experiments led him to incorrect interpretations.

We used field experiments to test the hypothesis that the anurans—*Rana cascadae, Bufo boreas,* and *Hyla regilla*—have differential sensitivity to ambient levels of UV-B radiation. We designed our field experiments so that factors vary naturally and simultaneously between experimental and control treatments, except the variable of interest—levels of UV-B radiation.

Licht stated that our field experiments "are of major concern," because "eggs placed in fully exposed very shallow water devoid of vegetation or protective substrate debris, might face abnormally intense levels of ultraviolet radiation." As stated in our original article, all of our experiments were conducted at natural oviposition sites. That is, apart from placing enclosures at each site, no modifications of the natural environment were made. Thus, the water color, depth of egg placement, substrate, and vegetation were all natural in our experiments. Moreover, we chose our three test species because they all characteristically lay their eggs in open, shallow water, exposed to UV-B radiation. Licht's objections are thus irrelevant.

Licht stated that there was a "startling difference" in temperatures between *treatments* (emphasis added). He noted that at one site, *H. regilla* developed at 21.5°C whereas at another site *R. cascadae* developed at 9.0°C. Consequently, he argued that *Hyla* was subjected to less UV-B radiation than *R. cascadae*, thus explaining why *Hyla* embryos showed little damage by UV-B radiation compared with the other species. Licht overlooked the experiment in which both *H. regilla* and *R. cascadae* were tested in the same lake at approximately the same temperatures (15.2–15.8°C). He also ignored the observation that at one site *Bufo* developed at higher temperatures (19.2–19.6°C) than did *Hyla* at another site (15.6–15.8°C). Again, Licht failed to comprehend our design and analyses that considered within species comparisons only, and he erroneously believed that there were differences in variables among treatments. What Licht calls between-treatment differences are actually between-lake differences.

In our study, eggs were placed in enclosures in a randomized block design, a method routinely used by ecologists. Indeed, our *PNAS* article (Blaustein et al. 1994a) was recently used as an ideal example illustrating this design in a statistics text (Ramsey and Shafer in press). This design allows experimental and control treatments to be conducted simultaneously, side by side, after randomly assigning enclosures to positions along the shore. Each block consisted of three treatments (not two as stated by Licht): enclosures open to natural sunlight including UV-B; covered with a UV-B blocking filter; or covered with a filter that transmitted UV-B (a control for placing a filter over eggs). Each block was replicated four times. To ensure that our results were not unique to a specific site, each species was tested at two sites: one site where only one species was found and another site where all three species were found.

There were no differences in variables such as temperature (recorded daily) among treatments. Therefore, regardless of treatment, embryos of a particular species were all subjected to the same natural variables except levels of UV-B radiation.

Embryos of *R. cascadae* and *B. boreas* displayed greater mortality in the two treatments that transmitted UV-B radiation compared with the treatment that shielded embryos from UV-B. The mortality rates of *H. regilla* embryos did not differ among treatments. The same results were observed at all sites. Thus, in our experiments, *Hyla* embryos survived well and *Rana* and *Bufo* poorly, regardless of temperature or site.

Licht worried that hatchlings may have swum out of, or potential predators may have invaded, enclosures. Neither of these occurred. Our enclosures were positioned on poles so that the tops barely broke the water surface. Furthermore, all larvae were counted in every enclosure every day, and all were accounted for. Licht also wondered if there were abnormalities in development. Although irrelevant to our analysis, growth patterns and abnormalities were recorded and are to be reported elsewhere.

Licht was puzzled that *R. cascadae* and *B. boreas* have lower levels of hatching success than *H. regilla* even under conditions where UV-B was removed implying that other factors may be involved in egg mortality. Many factors influence the hatching success of an amphibian. Some species have greater hatching success than others. This characteristic of a species' life history has been shaped over evolutionary time. The fact that *Hyla*, *Rana*, and *Bufo* show different natural hatching success rates is irrelevant to our experiments, because we used within species comparisons.

Contrary to Licht, we have never stated in any publication that there was a direct link between UV-B radiation and amphibian population declines. The title and discussion of our *PNAS* article (Blaustein et al. 1994a) clearly conveys our message that there may be a link between UV-B radiation and amphibian population declines. We have repeatedly stated that UV radiation cannot be the single explanation of amphibian declines and that habitat destruction is probably the major cause (e.g., Blaustein 1994). Indeed, some species (e.g., *H. regilla*) have behavioral, developmental, or biochemical adaptations that make them less susceptible to UV-B than others (Blaustein et al. 1994a). We have also stated that various agents acting alone or in concert may contribute to amphibian egg mortality (e.g., Blaustein 1994, Blaustein et al. 1994b). UV-B radiation seems to be one agent affecting certain species that lay their eggs in open shallow water. We have no idea how egg mortality may affect amphibians at the population level.

Our results were an initial contribution. We have other articles in press (e.g., Blaustein et al. in press) and in review that corroborate our results and that may provide additional clues to the puzzle of declining amphibian populations.

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