# Letters

## FIELD EXPERIMENTS, AMPHIBIAN MORTALITY, AND UV RADIATION

Lawrence E. Licht ("Amphibian decline still a puzzle," *BioScience* 46: 172–173) again criticizes our article on amphibians and ultraviolet (UV) radiation (Blaustein et al. 1994). This is the fourth similar criticism he has published about our article. Three were not peer reviewed (including two in *BioScience*), and one criticism was embedded in a discussion of his only data paper on the subject (see below). He has submitted at least one other critique of our work that was not published. Licht also criticized our work in public at the December 1995 meeting of the American Society of Zoologists in Washington, DC. We consider so many virtually identical attacks, by one person, about one article, to have overstepped the bounds of reasonable scientific discourse. Unfortunately, we are forced to respond to Licht's latest assault because he misrepresents our work, takes many of our statements out of context, and presents a misleading picture to the scientific community. Furthermore, he still fails to comprehend experimental design, proper statistical analysis, and the power of field experiments. In his latest criticism, he also shows that he does not know the natural history of the species in our study, two of which he says he is "familiar with."

In our initial study (Blaustein et al. 1994), we tested the hypothesis that the embryos of amphibians have differential sensitivity to ambient levels of UV-B radiation. There were two portions to the study: an assay of levels of photolyase, an enzyme that repairs UV-induced damage to DNA; and the use of field experiments to test the hatching success of embryos of three species of frogs and toads (*Rana cascadae, Bufo boreas*, and *Hyla regilla*) under varying conditions of light.

We designed our field experiments so that factors varied naturally and simultaneously between experimental and control treatments, except for the variable of interest—levels of UV-B radiation. In our study, eggs were placed in enclosures in a randomized block design, a method routinely used by ecologists. 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 in his original critique): enclosures open to natural sunlight, including UV-B; covered with a mylar UV-B blocking filter; or covered with an acetate filter that transmitted UV-B (a control for placing filters 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, including one site where all three species were found.

In our study, 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 site. Licht criticizes us for using hatching success as the "sole criterion" to assess UV-B damage to eggs. This criterion was used because it is easily quantifiable and lends itself to precise experimentation.

Although we were careful in describing how we collected eggs for each portion of our study, Licht takes our methods out of context to suggest that we took eggs from various regions and moved them to distant lakes for field experiments. We did collect eggs from several species from various regions *for the photolyase assay,* but we did not do this for the field experiments. Our egg collection techniques for the field experiments, according to Licht (p. 172) imply that "enclosures were placed...exactly where all three species, *Bufo boreas, Hyla regilla*, and *Rana cascadae*, lay their eggs." That is true. As stated in our original article, all of our experiments were conducted at natural oviposition sites. Enclosures were erected among egg masses where they were laid. Furthermore, apart from placing enclosures at each site, no modifications of the natural environment were made. 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 questions our statement that the three test species we have worked with for years lay their eggs in shallow water because he is "familiar with *B. boreas* and *H. regilla*" from his "work in British Columbia, and in that locality eggs are laid at varying depths...with only a small percentage of a population using such

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shallow water." Licht states (p. 172) it "is surprising that in Oregon all three species spawn in identical places." Well, that is how they spawn, and there are dozens of references on this phenomenon, including several in Table 2 of our original *PNAS* article, which he ignored. For example, Nussbaum et al. (1983) state that *H. regilla* lays its eggs in "shallow pools," and they observed *B. boreas* mating in "shallow inlets." Stebbins (1954) states that *H. regilla* lays its eggs "in shallow, quiet water" and that eggs are "occasionally found floating free at the surface," and *B. boreas* eggs are "ordinarily laid in shallow water." Nussbaum et al. (1983) state that *R. cascadae* eggs are laid in such shallow water that some egg masses "may be exposed partially to air." In the locality where Licht studied *B. boreas* and *H. regilla*, only a few individuals may lay their eggs in shallow water as he claims. However, even in British Columbia this does not seem to be the general situation where *B. boreas* "congregate in small ponds or pools to breed. They prefer shallow water with a sandy bottom" (Green and Campbell 1984, p. 69); *H. regilla* generally breed in "shallow, weedy, permanent ponds and swamps" (Green and Campbell 1984, p. 73). Perhaps Licht was studying aberrant populations in British Columbia.

Licht (p. 172) sums up our work by stating, "Interspecific comparisons in sensitivity to UV-B in the entire field study are tenuous given the problem with temperature controls." As we have stated previously, we did not make interspecific comparisons. We only compared the hatching success among the three treatments (UV-B blocking and two controls) in one species at one lake at one time.

Licht's own words can be used to demonstrate that he does not understand many aspects of experimental ecology. He states (p. 172), "I also understand an attempt was *made to control all variables but UV-B transmission* (our emphasis), yet unfortunately, temperature was not controlled and remains a major problem." Controlling all variables is precisely what we *did not do* and *what is not done* in a field experiment. We let everything vary naturally, including temperature, and controlled the one variable we were concerned with—UV-B radiation. The beauty of a field experiment is that everything varies naturally except the variable you are attempting to study.

Temperature was not a factor. There were no differences in unmanipulated variables such as temperature among treatments. Therefore, regardless of treatment, embryos of a particular species were all subjected to the same natural variables (including temperature) except levels of UV-B radiation. Likewise, his statement that varying climatic conditions could have affected our results is irrelevant. When it was sunny, all treatments within a species at one lake were exposed to equivalent amounts of sunlight. When it was cloudy, all treatments were under equivalent cloud cover.

Licht suggests that we removed eggs from their jelly matrix and that the jelly matrix may protect eggs from UV-B. We did not remove eggs from their jelly, and eggs in all treatments were treated the same, yet eggs of *B. boreas* and *R. cascadae* had a higher hatching success under treatments that filtered out UV-B. Licht should also know that both *B. boreas* and *R. cascadae* have much thicker jelly coats than eggs of *H. regilla*, whose eggs are not impaired by UV-B. But *H. regilla* has more photolyase activity than those two species, which probably helps protect it from UV-B.

Even though we have now conducted dozens of experiments at many sites for several years on several species using similar methods (Licht fails to cite our other papers, such as Blaustein et al. 1995a, Kiesecker and Blaustein 1995), Licht observed that at one site, in one year, the eggs of one species, *R. cascadae*, appeared to have higher mortality under the acetate control than in open sunlight (Blaustein et al. 1994). He fails to mention that this difference was not significant.

Licht states (p. 173), "They believe I failed to understand their statistical analysis." That is true. He admonishes us for not testing assumptions of the ANOVA we conducted and that it may have been necessary for us to use an arcsine transformation. We *did* test the assumptions (normality, independence, variance, homogeneity) of the pooled data, and our data *did not* violate any of the assumptions of the statistical analyses, so transformation was not necessary (Zar 1984). Although our design and analyses were good enough to be used as an ideal example in a statistics text, in his critique Licht mocked this fact without a sound statistical basis to do so.

Licht warns us to be careful in future interviews with the press because one reporter wrote that we said we were the first to directly link a specific cause, other than habitat destruction, to amphibian declines. We did not say this. As we all know, press reports vary in their adherence to the facts, and information providers often have little control over the accuracy of reporting. For example, we also did not provide the information to the reporter who wrote that frogs from Oregon were being taken aboard space craft by aliens (Stern 1990). Contrary to what Licht states, we have never stated in any publication that there was a direct link between UV-B and amphibian declines. In fact, we have made many cautionary statements such as, "Obviously UV-B cannot be invoked as an explanation for declines of all amphibian species" (Blaustein et al. 1995a, p. 742) and "UV-B is less likely to affect species that lay their eggs in relatively deep water or under dense foliage..." (Kiesecker and Blaustein 1995, p. 11051) and at this point "We have no idea how egg mortality may affect amphibians at the population level" (Blaustein et al. 1995b, p. 515). We believe that multiple factors are involved in causing embryo mortality in Oregon that could *possibly* lead to amphibian population declines (Kiesecker and Blaustein 1995). We are open-minded about the effects of UV-B on amphibians. For example, we have suggested that the decline in red-legged frogs (*Rana aurora*) in Oregon was probably not due to UV-B radiation (Blaustein et al. in press). We have repeatedly stated that some species (e.g., *H. regilla* and *R. aurora*) have behavioral, developmental, or biochemical adaptations that make them less susceptible to UV-B than others.

Licht's persistent criticisms of our work also lack credibility because his own work on the subject is seriously flawed and shows his poor knowledge of experimental ecology. For example, Grant and Licht (1995; cited in Licht's critique of our work in *BioScience*) failed to include replicates for several experiments. Further, control and experimental treatments differed in more ways than just the treatments. In addition to differing levels of UV-B, treatments differed in numbers of eggs and time of exposure to UV-B. In fact, in one experiment there was simultaneous manipulation of different exposure time and whether embryos were shielded or not. What was termed *ecologically relevant* UV doses were based on one measurement in summer of 1991. These dose levels were then used for all tests from 1992–1994. The *maximum* dosage of UV received in their study is the equivalent of four hours per day for five days. Embryos in our treatments were subjected to natural doses of UV throughout the day, for their entire developmental period (often weeks). Grant's and Licht's statistical analysis was flawed in several ways. For example, their design measured the response of several variables, thus dictating use of MANOVA (Multivariate Analysis of Variance) in analysis or alternatively, use of multiple ANOVAs using adjusted significance levels. Instead, they used multiple ANOVAs without adjusting $P$ values, leading to an inflation of Type I error.

Licht seems so concerned with criticizing the results of our work that he seems to have lost his objectivity and fails to see how his own data possibly show effects that could potentially contribute to a population decline. For example, in one experiment, embryos dosed with *less* than the highest measured ambient levels of UV-B had lower hatching success than controls receiving no UV-B, and developmental abnormalities due to UV-B were common throughout the study (Grant and Licht 1995). Unfortunately, design and analysis flaws do not allow us to make strong conclusions on how UV affected amphibians in his study.

We hope that this regrettable discourse is concluded. Although scientific progress depends on skepticism and critical evaluation, we believe that further discussion of these particular studies would contribute little to progress. Instead, we intend to spend our time pursuing through scientific means, the possible influence of UV and other agents on amphibian populations, an issue which is by no means resolved. We hope Licht chooses a similar course of endeavor.

ANDREW R. BLAUSTEIN
JOSEPH M. KIESECKER
*Department of Zoology*
*Oregon State University*
*Corvallis, OR 97331-2914*

SUSAN C. WALLS
*Department of Biology*
*City College of New York*
*Convent Ave. at 138th St.*
*New York, NY 10031*

D. GRANT HOKIT
*Department of Wildlife Ecology and Conservation*
*University of Florida*
*Gainesville, FL 32611-0430*

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