DNA Repair Activity and Resistance to Solar UV-B Radiation in Eggs of the Red-legged Frog

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Abstract: *We assessed DNA repair and resistance to solar radiation in eggs of the red-legged frog* (Rana aurora)*, a species whose populations appear to be in decline. Specifically, we measured the activity of photoreactivating enzyme, photolyase, in* R. aurora *oocytes. In some species photoreactivation is the most important mechanism for repair of UV-damaged DNA. We also compared the hatching success of* R. aurora *eggs subjected to ambient levels of UV-B radiation with those shielded from UV-B radiation. We found photolyase levels in* R. aurora *to be relatively high when compared with other amphibians and hatching success to be unaffected by UV-B radiation. We suggest that UV-B radiation is an unlikely cause for declining populations of red-legged frogs.*

Reparación de ADN y resistencia a radiación solar UV-B en huevos de rana de piernas rojas

Resumen: *Evaluamos la reparación de ADN y la resistencia a la radiación solar en huevos de rana de piernas rojas,* Rana aurora*, una especie cuyas poblaciones aparentemente están declinando. Específicamente, medimos la actividad de la enzima fotoreactivante, fotoliasa, en ovocitos de* R. aurora*. En algunas especies la fotoreactivación es el mecanismo más importante en la reparación de ADN dañado por rayos ultravioleta (UV). También comparamos el éxito de eclosión de huevos de* R. aurora *sujetos a niveles ambientales de radiación UV-B con el de huevos protegidos contra dicha radiación. Encontramos que los niveles de fotoliasa en* R. aurora *son relativamente más altos que en otros anfibios y que el éxito de eclosión no es afectado por la radiación UV-B. Sugerimos que la radiación UV-B no es un factor en la declinación de poblaciones de ranas de piernas rojas.*

Introduction

An unprecedented loss of the variety and numbers of species around the world is occurring (McNeely et al. 1990; Wilson 1992) as part of an overall "biodiversity crisis." In particular, numerous recent reports suggest that many species within the class amphibia are undergoing population declines and range reductions (e.g., Barinaga 1990; Blaustein & Wake 1990, 1995; Blaustein et al. 1994*c*; Crump et al. 1992; Pounds & Crump 1994; Tyler 1991; Reed & Blaustein 1995; Richards et al. 1993; Wake 1991).

Habitat destruction is probably the most significant cause of amphibian population declines (Blaustein 1994; Blaustein & Wake 1995; Pechmann & Wilbur 1994). Some declines may reflect temporary population fluctuations (Blaustein 1994; Pechmann & Wilbur 1994; but see Reed & Blaustein 1995). However, the causes of many amphibian population declines are unknown (e.g., Crump et al. 1992; La Marca & Reinthaler 1991; Pounds & Crump 1994; Richards et al. 1993). This seems to be especially true for many species of ranid frogs in western North America (e.g., Corn & Fogleman 1984; Hayes

Paper submitted October 26, 1995; revised manuscript accepted January 17, 1996.

& Jennings 1986; Fellers & Drost 1993; McAllister et al. 1993). For example, why the red-legged frog *(Rana aurora)* has disappeared from a large portion of its historical range in Oregon and California (Moyle 1973; Nussbaum et al. 1983; Hayes & Jennings 1986; Blaustein & Wake 1990) is unknown. *R. aurora* is a candidate for threatened or endangered status at the federal level in the United States (Federal Register 1991). In the Willamette Valley of Oregon breeding populations of *R. aurora* are much rarer than they have been historically (Nussbaum et al. 1983).

Some clues to the declines of certain amphibian populations have recently emerged. For example, in some regions of western North America the eggs of certain amphibian species have experienced high mortality at natural oviposition sites that could contribute to population declines (e.g., Blaustein et al. 1994*a, b*). At least two factors have contributed to egg mortality in the Cascades frog *(Rana cascadae)* and western toad *(Bufo boreas)* in Oregon: a pathogenic fungus (Blaustein et al. 1994*b*) and ultraviolet-B (UV-B; 290–320 nm) radiation (Blaustein et al. 1994*a*; Kiesecker & Blaustein 1995). Unlike *R. cascadae* and *B. boreas*, there are no reports of massive egg mortality in *R. aurora*. No experimental tests of key hypotheses regarding the declines of *R. aurora* populations have been reported.

Because of the potentially lethal effects of UV-B radiation on amphibian eggs and embryos (Blaustein et al. 1994*a*; Blaustein et al. 1995; Kiesecker & Blaustein 1995; Long et al. 1995; Worrest & Kimeldorf 1975), we examined (1) the ability of *R. aurora* to repair UV-damaged DNA in eggs and (2) the resistance of *R. aurora* eggs to ambient levels of UV-B radiation. Specifically, we measured the activity of photoreactivating enzyme, photolyase, in *R. aurora* oocytes by a biochemical assay. In some organisms photoreactivation is the most important mechanism for repair of cyclobutane pyrimidine dimers (CPDs), which are major cytotoxic and mutagenic photoproducts in DNA (Pang & Hays 1991). UV photoproducts impede gene expression by blocking transcription and can kill cells by interfering with DNA replication. Higher levels of photolyase activity would be expected to mediate efficient repair of UV damage to DNA in eggs (Blaustein et al. 1994*a*). In the field we measured hatching success of embryos in enclosures that transmitted or blocked UV-B radiation.

Methods

Oocyte Extract for Photolyase Activity Assay

Using methods similar to those described in Blaustein et al. (1994*a*) we collected oocytes from *R. aurora* from a pond in Linn County, Oregon (about 3.5 km southeast of Corvallis, Oregon, U.S.A.). Ovaries were dissected and ovary lobes were sectioned and dounced with 3× volume of Modified Transcription Buffer (Blaustein et al. 1994*a*; Glikin et al. 1984). The resulting slurry was centrifuged in a Beckman TLA 110.2 rotor at 4°C for 60 minutes at 60,000 × g. The exudate layer between the debris pellet and upper yolk layer was recovered and used for photolyase and protein assays (Blaustein et al. 1994*a*). Protein concentrations were determined by the Bradford technique (Bradford 1976). Yields were about 2 mL of extract at 11.8 mg/mL from about 400 oocytes.

The stock extract was diluted to 0.3 mg/mL in Modified Transcription Buffer and assayed for blue-light dependent removal of CPDs from exogenous UV-irradiated DNA by acid hydrolysis and thin-layer chromatography, as described by Blaustein et al. (1994*a*). Assays with 1.5, 3.0, 4.5, and 6.0 μg total extract protein respectively defined the linear range of response for the assay. Results from duplicate assays at each protein concentration were averaged to establish the specific activity of the *R. aurora* oocyte extract expressed as CPDs removed per hour per μg extract protein (Blaustein et al. 1994*a*).

Field Experiments

Field experiments were conducted in an area of the Willamette Valley of Oregon where *R. aurora* was historically abundant but is now rare (Nussbaum et al. 1983).

We collected clutches of *R. aurora* eggs from a site in Benton County, Oregon (approximately 10 km south of Corvallis, Oregon, U.S.A.). Tests were conducted at the Lewis Brown Horticultural Farm about 8 km southwest of Corvallis (Benton Co.; 76 m elevation). We placed 150 newly deposited eggs (25 eggs from each of six clutches, <24 hr old) with their jelly matrix intact in each of 12 enclosures (38 × 38 × 7 cm). Enclosures were placed within small plastic pools (110 cm diameter, 18 cm deep). Within the pools enclosures with eggs were immersed in 5–10 cm of well water, a depth at which eggs are often laid (Stebbins 1954; personal observations). The 12 enclosures were assigned to three sunlight treatments: unfiltered sunlight, sunlight filtered to remove UV-B and shorter wavelengths, and sunlight filtered to remove wavelengths shorter than about 290 nm (a control for placing filters over eggs). There were four replicates per treatment (Fig. 1). Enclosures were placed in a linear array in a randomized block design. Enclosures had clear plexiglass frames with floors of 1 mm^2 fiberglass mesh screen.

A UV-B blocking filter (50 × 50 cm) made of mylar was placed over one-third of the enclosures. An acetate filter (50 × 50 cm) that transmitted UV-B but not UV-C (wavelengths less than about 290 nm) was placed over another third of the enclosures. The remaining enclosures had no filters. The mylar blocked 100% of UV-B (290–320 nm). The acetate allowed about 80% transmis-

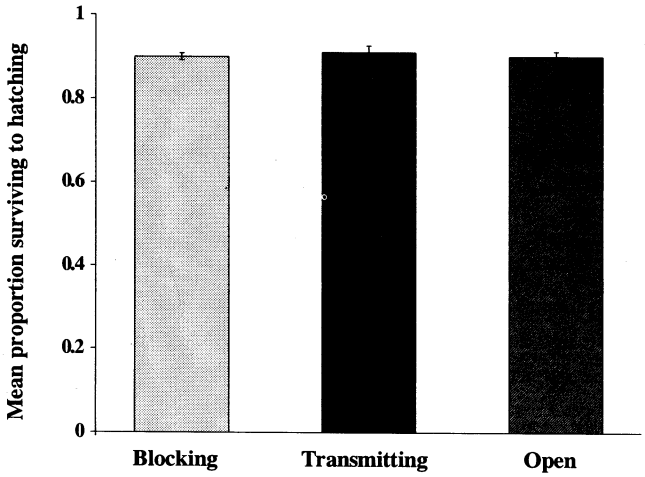

*Figure 1. Effects of UV-B radiation on hatching success ($\bar{X} \pm SE$) in red-legged frog (*Rana aurora*) embryos. There were three treatments: one set of enclosures had UV-B blocking filters, one set had UV-B transmitting filters, and a third set had no filters. Each enclosure had 150 eggs. Each treatment was replicated four times for a total of 12 enclosures.*

sion of UV-B (Blaustein et al. 1994*a*). The transmitting properties of the mylar and acetate were assessed by scanning a UV-B 313 lamp directly with an Optronics 752 spectroradiometer and comparing the transmission with the same lamp covered by mylar or acetate.

The experiment was terminated when all the original embryos either hatched or died. All eggs were counted each day during the experiment. Survival was measured as the proportion of hatchlings produced per enclosure. Daily temperatures (°C) were taken within enclosures in each treatment. Tests were conducted from 27 February to 19 March 1994. Analysis of variance (ANOVA) was used to test for treatment effects on embryo survivorship. We used a power test (Cohen 1988; Reed & Blaustein 1995) to provide statistical confidence in our results. Power values below 0.8 are considered inadequate for confidence in concluding that there were no differences between treatments.

Results

Red-legged frog oocytes had relatively high levels of photolyase activity, averaging 6.09×10^{11} CPDs per hour per μg (Table 1; Blaustein et al. 1994*a*).

In the field a preliminary analysis indicated no significant block effects. Therefore, the block and error terms were pooled for remaining tests. The eggs in field experiments were not affected by ambient levels of UV-B radiation. There were no significant differences in hatching success among the three regimes (Fig. 1; Table 2; power = 0.968). All eggs were accounted for during the experiment. Predation of eggs did not occur.

ANOVA revealed no significant temperature differences between treatments ($F_{2,9} = 0.059$; $p = 0.943$). Mean temperatures were 18.9°C, 18.7°C, and 18.8°C for the unfiltered, UV-B transmitting, and UV-B-blocking regimes respectively.

Discussion

Compared with other amphibians *R. aurora* has relatively high levels of photolyase activity (Table 1). It has more than four times the activity found in western toad *(B. boreas)* and more than twice the activity found in Cascades frog *(R. cascadae)* eggs (Blaustein et al. 1994*a*). Of 10 North American amphibian species we have examined for photolyase activity, only Pacific treefrogs *(Hyla regilla)* have higher levels (Blaustein et al. 1994*a*). Similar to *H. regilla* (Blaustein et al. 1994*a*), *R. aurora* eggs were not affected by ambient levels of UV-B radiation in any of the three regimes.

Although there are no data for increasing UV-B at our specific study site and no long-term data for UV-B levels in the United States, measurements at Toronto, Canada,

Table 1. Population status, photolyase levels (from eggs and oocytes), and resistance to UV-B in five species of amphibians for which field experiments have been performed.

Species	*Activity of photolyase 10^{11} CPDs per hr per μg (± SE)*	*Hatching success in field experiments*	*Population status*
Hyla regilla Pacific treefrog	7.5 (0.35)	hatching success not affected by UV-B	no reported declines
Rana aurora Red-legged frog	6.09 (0.22)	hatching success not affected by UV-B	declining
Rana cascadae Cascades frog	2.4 (0.23)	greater in UV-B-blocking regimes	declining in California and loss of populations in Oregon
Bufo boreas Western toad	1.3 (0.08)	greater in UV-B-blocking regimes	declining
Ambystoma gracile Northwestern salamander	1.0 (0.10)	greater in UV-B-blocking regimes	unknown

Table 2. Univariate analysis (ANOVA) of hatching success in *Rana aurora* embryos.*

Source of variation	MS	df	F	p
Treatment	1.120	2	0.174	0.843
Error	6.436	9		

*MS = *mean square;* df = *degrees of freedom;* F = *F statistic; and* p = *probability.*

which is at the same latitude (44°N) as our experimental site, showed a 35% increase in UV-B per year in winter and a 7% increase per year in summer since 1989 (Kerr & McElroy 1993). These measurements were apparently caused by a downward trend in total ozone that was measured at the same time in Toronto. However, these data may not reflect values that will continue into the future (Kerr & McElroy 1993). Trends from a few years of data may be influenced by natural cyclic variations (Kerr & McElroy 1993).

It is generally assumed that UV levels are greater at higher altitudes (Blumthaler & Ambach 1990; Blumthaler 1993). Nevertheless, the relatively high levels of photolyase suggest the possibility of strong selective pressures for UV-B resistance in *R. aurora* at the lower elevations where we conducted our tests. Why does *R. aurora* have such high levels of photolyase?

R. aurora has ecological characteristics that may expose its eggs to prolonged, low levels of solar radiation. In Oregon and California, *R. aurora* lays its eggs in open shallow water and the egg masses often float to the surface or rise above the surface as water evaporates (Stebbins 1951; Storm 1960; Nussbaum et al. 1983). In western Oregon the developmental time to hatching is prolonged, probably because *R. aurora* lays its eggs in winter, as early as January in relatively cold (often 6–7°C) water (Brown 1975; Nussbaum et al. 1983; Stebbins 1954). *R. aurora* embryos may take 6 weeks to hatch in nature (Nussbaum et al. 1983; Storm 1960). In comparison, *R. cascadae*, a species with low photolyase levels, has a shorter developmental time than *R. aurora* (Sype 1975). Thus, compared with *R. cascadae, R. aurora* eggs may be subjected to prolonged bouts of lower levels of UV-B radiation. Moreover, under similar experimental regimes to those described for *R. aurora*, northwestern salamander *(Ambystoma gracile)* eggs had reduced hatching success under UV-B-transmitting regimes compared with those under UV-B-blocking regimes at low elevation (183 m; Blaustein et al. 1995). The eggs of *A. gracile* have relatively low photolyase activity levels (Blaustein et al. 1994*a*; Table 1). Thus, exposure to solar radiation even at low elevations can potentially kill significant fractions of amphibian eggs with relatively poor capacities to repair UV-damaged DNA.

Our results are consistent with our previous hypothesis that amphibian eggs with relatively high species-specific levels of photolyase are more resistant to UV-B damage than those with lower levels of photolyase (Blaustein et al. 1994*a*; Table 1). The fact that levels of UV-B radiation are most likely relatively low at our study sites, that levels of photolyase activity are high, and that there have been no reports of abnormal mortality of eggs together suggest that UV-B radiation is not playing a major role in the decline of *R. aurora* populations. It is possible, however, that UV-B radiation may affect *R. aurora* as larvae or after metamorphosis. There may also be interactions between UV-B radiation and other factors that could contribute to embryo mortality (Kiesecker & Blaustein 1995). In those species, unlike *R. aurora*, with relatively low photolyase activity, the detrimental effects of UV-B radiation may only become manifest if such an interaction occurs (Kiesecker & Blaustein 1995). For example, UV-B radiation may compromise the disease defense systems of eggs and embryos making them more susceptible to pathogens (Blaustein et al. 1994*b*; Kiesecker & Blaustein 1995). We suggest, however, that introductions of exotic species such as bullfrogs *(Rana catesbeiana)*, pollution, or habitat alteration are more likely causes for the decline of populations of *R. aurora* (Blaustein 1994; Moyle 1973; Nussbaum et al. 1983) than UV-B radiation. The UV-B radiation is probably most detrimental to those species that not only lay their eggs in open, shallow water, but also have poor capabilities to repair UV-damaged DNA.

Acknowledgments

We thank Cheri Miller and Roxanne Gamiao for their help, Bruce Menge for advice on statistical analyses, the anonymous reviewers for their comments, and NSF for funding (DEB-9423333).

Literature Cited

Barinaga, M. 1990. Where have all the froggies gone? Science **247:** 1033–1034.

Blaustein, A. R. 1994. Chicken Little or Nero's fiddle? A perspective on declining amphibian populations. Herpetologica **50:**85–97.

Blaustein, A. R., B. Edmond, J. M. Kiesecker, J. J. Beatty, and D. G. Hokit. 1995. Ambient ultraviolet radiation causes mortality in salamander eggs. Ecological Applications **5:**740–743.

Blaustein, A. R., P. D. Hoffman, D. G. Hokit, J. M. Kiesecker, S. C. Walls, and J. B. Hays. 1994*a*. UV repair and resistance to solar UV-B in amphibian eggs: a link to population declines? Proceedings of the National Academy of Sciences of the United States of America **91:**1791–1795.

Blaustein, A. R., D. G. Hokit, R. K. O'Hara, and R. A. Holt. 1994*b*. Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. Biological Conservation **67:**251–254.

Blaustein, A. R., and D. B. Wake. 1990. Declining amphibian populations: a global phenomenon? Trends in Ecology & Evolution **5:**203–204.

Blaustein, A. R., and D. B. Wake. 1995. The puzzle of declining amphibian populations. Scientific American **272:**52–57.

Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994*c*. Amphibian de-

clines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology **8:**60–71.

Blumthaler, M. 1993. Solar UV measurements. Pages 71–94 in M. Tevini, editor. UV-B radiation and ozone depletion: effects on humans, animals, plants microorganisms and materials. Lewis Publishers, Boca Raton, Florida.

Blumthaler, M., and W. Ambach. 1990. Indication of increasing solar ultraviolet-B radiation flux in alpine regions. Science **248:**206–208.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. Analytical Biochemistry **72:**248–254.

Brown, H. A. 1975. Reproduction and development of the red-legged frog, *Rana aurora*, in northwestern Washington. Northwest Science **49:**241–252.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences. 2nd edition. Lawrence Erlbaum, Hillsdale, New Jersey.

Corn, P. S., and J. C. Fogleman. 1984. Extinction of montane populations of the northern leopard frog *(Rana pipiens)* in Colorado. Journal of Herpetology **18:**147–152.

Crump, M. L., F. R. Hensley, and K. L. Clark. 1992. Apparent decline of the golden toad: underground or extinct? Copeia **1992:**413–420.

Federal Register. 1991. Endangered and threatened wildlife and plants: animal candidate review listing as endangered or threatened species proposed rule. Part VIII. U.S. Fish and Wildlife Service, Washington, D.C.

Fellers, G. M., and C. A. Drost. 1993. Disappearance of the Cascades frog *Rana cascadae*, at the southern end of its range, California. Biological Conservation **65:**177–181.

Glikin, G. C., I. Ruberti, and A. Worcel. 1984. Chromatin assembly in *Xenopus* oocytes: in vitro studies. Cell **37:**33–41.

Hayes, M. P., and M. R. Jennings. 1986. Decline of western frog species in western North America: are bullfrogs *(Rana catesbeiana)* responsible? Journal of Herpetology **20:**490–509.

Kerr, J. B., and C. T. McElroy. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science **262:** 1032–1034.

Kiesecker, J. M., and A. R. Blaustein. 1995. Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proceedings of the National Academy of Sciences **92:** 11049–11052.

La Marca, E., and H. Reinthaler. 1991. Population changes in *Atelopus* species of the Cordillera de Merida, Venezuela. Herpetological Review **22:**125–128.

Long, L. E., L. Saylor, and M. E. Soulé. 1995. A pH/UV-B synergism in amphibians. Conservation Biology **9:**1301–1303.

McAllister, K. R., W. P. Leonard, and R. M. Storm. 1993. Spotted frog *(Rana pretiosa)* surveys in the Puget Trough of Washington, 1989–1991. Northwest Naturalist **74:**10–15.

McNeely, J. A., K. R. Miller, W. V. Reid, R. A. Mittermeier, and T. B. Werner. 1990. Conserving the world's biodiversity. International Union for the Conservation of Nature, World Resource Institute, CI, World Wildlife Fund-U.S., The World Bank, Washington, D.C.

Moyle, P. B. 1973. Effects of introduced bullfrogs, *Rana catesbeiana*, on the native frogs of the San Joaquin Valley, California. Copeia **1973:**18–22.

Nussbaum, R. A., E. D. Brodie, Jr., and R. M. Storm. 1983. Amphibians and reptiles of the Pacific Northwest. The University Press of Idaho, Moscow.

Pang, Q., and J. B. Hays. 1991. UV-inducible and temperature-sensitive photoreactivation of cyclobutane pyrimidine dimers in *Arabidopsis thaliana*. Plant Physiology **95:**536–543.

Pechmann, J. H. K., and H. M. Wilbur. 1994. Putting amphibian decline populations in perspective: natural fluctuations and human impacts. Herpetologica **50:**65–84.

Pounds, J. A., and M. L. Crump. 1994. Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. Conservation Biology **8:**72–85.

Reed, J. M., and A. R. Blaustein. 1995. Assessment of "nondeclining" amphibian populations using power analysis. Conservation Biology **9:**1299–1300.

Richards, S. J., K. R. McDonald, and R. A. Alford. 1993. Declines in populations of Australia's endemic tropical rainforest frogs. Pacific Conservation Biology **1:**66–77.

Stebbins, R. C. 1951. Amphibians of western North America. University of California Press, Berkeley.

Stebbins, R. C. 1954. Amphibians and reptiles of western North America. McGraw-Hill, New York.

Storm, R. M. 1960. Notes on the breeding biology of the red-legged frog *(Rana aurora aurora)*. Herpetologica **16:**251–259.

Sype, W. E. 1975. Breeding habits, embryonic thermal requirements and embryonic and larval development of the Cascade frog, *Rana cascadae* Slater. Ph.D. thesis. Oregon State University, Corvallis.

Tyler, M. J. 1991. Declining amphibian populations—a global phenomenon? An Australian perspective. Alytes **9:**43–50.

Wake, D. B. 1991. Declining amphibian populations. Science **253:**860.

Wilson, E. O. 1992. The diversity of life. The Belknap Press, Cambridge, Massachusetts.

Worrest R. C., and D. J. Kimeldorf. 1975. Photoreactivation of potentially lethal UV-induced damage to boreal toad *(Bufo boreas boreas)* tadpoles. Life Sciences **17:**1545–1550.