

*Department of Zoology, Oregon State University, Corvallis; Department of Biological Sciences, University of Maine, Orono; Natural Science Division, Pepperdine University, Malibu and Department of Biology, Pennsylvania State University, University Park*

## **Effects of Ultraviolet Radiation on Locomotion and Orientation in Roughskin Newts (*Taricha granulosa*)**

Andrew R. Blaustein, Douglas P. Chivers, Lee B. Kats & Joseph M. Kiesecker

Blaustein, A. R., Chivers, D. P., Kats, L. B. & Kiesecker, J. M. 2000: Effects of ultraviolet radiation on locomotion and orientation in roughskin newts (*Taricha granulosa*). *Ethology* **106**, 227–234.

### **Abstract**

Environmental changes, including those associated with the atmosphere may significantly affect individual animals and ultimately populations. Ultraviolet (UV) radiation, perhaps increasing due to stratospheric ozone depletion, has been linked to mortality in a number of organisms, including amphibians. The eggs and larvae of certain amphibian species hatch at significantly lower rates when exposed to ambient ultraviolet light. Yet little is known about the sublethal effects of UV radiation. For example, UV radiation may affect specific behaviors of an animal that could alter its ability to survive. To examine if UV radiation affects amphibian behavior, we used roughskin newts (*Taricha granulosa*) as a model. Newts were exposed to low-level doses of UV in the laboratory and then tested in the field to examine if UV-exposed and control (no UV) newts differed in orientation towards water or in locomotor activity levels. UV-exposed and control newts both exhibited a significant orientation towards water in field tests but there was no significant difference in orientation between treatments. However, UV-exposed newts were significantly more active than control newts. Our results suggest that exposure to short-term low levels of UV radiation alters certain behaviors. Environmentally induced changes in behavior may have significant ecological and evolutionary consequences.

Corresponding author: Andrew R. Blaustein, Department of Zoology, Oregon State University, Corvallis, OR 97331–2914, USA. E-mail:blaustea@bcc.orst.edu

### **Introduction**

An increasing number of studies have addressed how specific environmental stresses (e.g. acid precipitation, ultraviolet-B radiation) affect mortality rates of

particular organisms (e.g. Harte & Hoffman 1989; Blaustein et al. 1998). However, few studies have addressed the sublethal effects of specific environmental stressors. For example, environmental stressors may not kill an animal but may significantly affect its behavior. Because many behaviors are associated with reproduction and foraging, behavioral changes may have significant ecological and evolutionary consequences. Yet, there is a lack of experimental evidence on how environmental stresses affect the behavior of animals in nature.

One hypothesis advanced for the mortality of amphibian embryos at several well-separated global locales is hypersensitivity to small increases in UV-B (280–315 nm) radiation at the earth's surface, which may have already occurred as a result of stratospheric ozone depletion (e.g. Blaustein et al. 1994, 1997, 1998; Anzalone et al. 1998; Lizana & Pedraza 1998; Broomhall et al., in press). Moreover, ambient UV-B radiation causes anatomical and physiological damage in larval and adult amphibians (Worrest & Kimeldorf 1975, 1976; Blaustein et al. 1997; Nagl & Hofer 1997; Fite et al. 1998). Thus, individuals that are not killed by exposure to ambient UV-B radiation may be profoundly affected as they receive sublethal doses of UV-B. These sublethal effects could influence behaviors that are critical for survival. For example, deformities of the body in larval salamanders due to exposure to ambient UV-B radiation may affect their ability to swim (Blaustein et al. 1997). UV-induced retinal damage in basking frogs may affect an individual's ability to capture prey or avoid predation (Fite et al. 1998). Yet, there is very little experimental evidence on how sublethal doses of UV-B radiation may affect behavior.

In this study, we report the effects of UV-B exposure on orientation and locomotor activity in the roughskin newt (*Taricha granulosa*). Activity patterns, orientation and homing are behaviors that are critical to amphibians. Many species of amphibians return to the same sites to breed each year and most amphibians need to find water for breeding and egg laying (Duellman & Trueb 1986; Mason et al. 1998). Thus, any factor that hampers orientation behavior and activity patterns could reduce an individual's fitness. Moreover, roughskin newts are ideal for such a study because they often float near the surface of open water and live in shallow streams and are thus exposed to long bouts of solar radiation (Nussbaum et al. 1983; Blaustein et al. 1995). Thus, potential increases in UV-B radiation that may have come about with stratospheric ozone depletion could have significant sublethal effects on *T. granulosa* that may be manifest by changes in behavior.

Several studies have shown that when newts are stressed, their locomotor activity patterns may change (e.g. Moore et al. 1984; Lowry & Moore 1991). Depending upon the circumstances, locomotor activity may increase or decrease when newts are under stress. We tested the hypothesis that locomotor activity patterns and orientation behavior would change when newts were stressed by UV-B radiation.

## Methods

In Oct. 1996 we collected 84 adult male roughskin newts from the Corvallis Watershed Reservoir (Benton County, USA; 44° 30' N, 123° 30' W; elev-

ation = 183 m; 4047 surface ha) in the Oregon Coast Range and transported them to Oregon State University. In the laboratory we placed six newts into each of 14 glass aquaria (30 × 20 × 25 cm) that were filled with dechlorinated tap water to a depth of 5 cm. Newts remained in the water; climbing material was not available in the aquaria. The 14 tanks were placed in a room that was illuminated with artificial UV light on a 14:10 L:D photoperiod using Q-Panel UVB313 lamps (Hays et al. 1996). Lamps were checked weekly for output. Room temperature was maintained at about 13°C.

Most UV radiation of biological concern is in the 280–315 (UV-B) nm band (Tevini 1993; Blaustein et al. 1994); critical biomolecules absorb light of higher wavelength less efficiently and stratospheric ozone absorbs most light of lower wavelength. To remove UV-B and UV-C (200–280 nm), but not UV-A (320–400 nm) seven tanks were covered with 12 mm glass (cut-off at about 320 nm; UV-B Blocking treatment, Fig. 1). To remove UV-C (and a small amount of UV-B) seven aquaria were covered with 3 mm glass (cut-off at about 295 nm; UV-B exposed treatment, Fig. 1). UV-B and UV-A intensities under glass shields were much less than mid-day midsummer solar intensities at 45°N latitude where field experiments were performed (Fig. 1; Hays et al. 1996). Of course, solar values will be much less at other times of the day and about 10% as much in Dec. as in Jun.

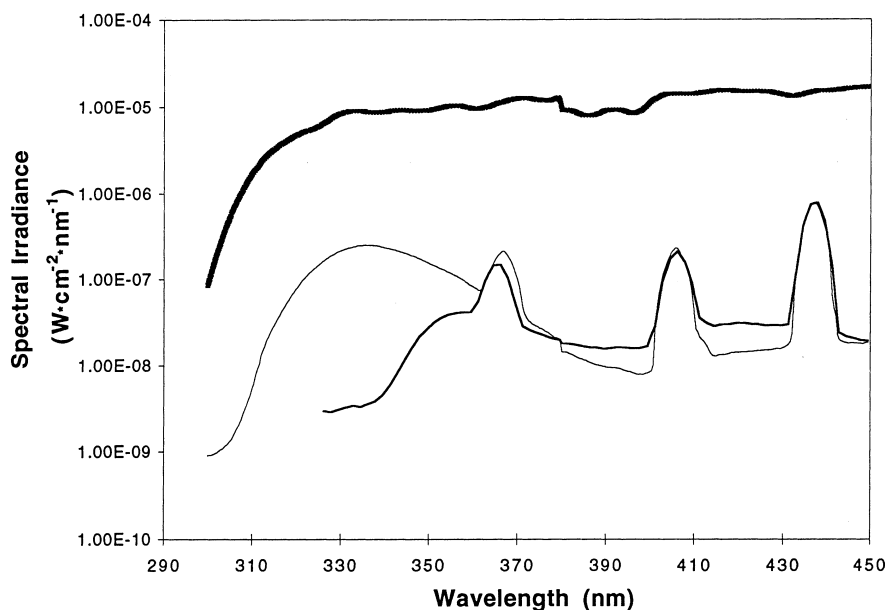


Fig. 1: Spectra of artificial and solar light sources. An International Light IL 1700 spectroradiometer was used to measure the irradiance of the bank of Q-Panel UVB313 lamps to which experimental subjects were exposed. Heavy solid upper line is solar irradiance on a clear day at Corvallis, Oregon (latitude 45°N) on 6 Jun. 1996 at solar 13:00 h. Light line is irradiance under lamps shielded by 3 mm glass. Lower heavy line is irradiance of lamps shielded by 12 mm glass

UV-blocking and UV-exposed tanks were arranged in pairs. Newts were kept in the laboratory under UV light for 14 d and were fed earthworms ad libitum.

After laboratory UV exposure we transported the newts back to the Corvallis Watershed Reservoir to test for differences in activity and (or) orientation between those in the UV-blocking and UV-exposed treatments. All tests were completed on a single day between 11:00 and 15:00 h. We identified three areas on land suitable for completing the tests, each consisting of a large flat sparsely vegetated area in which we marked (by etching in the ground) a test circle 6 m in diameter. The circles were 30.7, 42.5 and 51.2 m from the shoreline. The most direct path to the water was at a compass direction of 240, 255 and 255 degrees, respectively. We could not complete the experiments at other locations around the reservoir because of uneven terrain.

We started each trial by placing an individual newt, whose initial position was random with respect to the water, under an opaque cover (an inverted plastic pail) in the center of the 6-m diameter circle. After a 30-s acclimation period, we removed the cover via a remote pulley system and recorded: (1) time taken for the newt to start walking, (2) time taken for the newt to leave the circle, and (3) the location (compass direction) where the newt left the circle. Observers were positioned at least 5 m from the outside circle in random positions throughout testing.

We used t-tests (two-tailed) to compare the UV-blocking and UV-exposed treatments with respect to both time taken to initiate movement and time taken to leave the circle. We used separate Watson U<sub>2n</sub> tests (Batschelet 1981) to determine whether the orientation of the newts in either the UV-blocking or UV-exposed treatments was significantly different from random. A Watson U<sub>2</sub> test (Batschelet 1981) was used to test for differences between the UV-blocking and UV-exposed treatments with respect to orientation.

## Results

It took  $48.7 \pm 9.5$  s ( $\bar{x} \pm$  SE) for UV-exposed newts to initiate movement following removal of the opaque cover compared to  $85.9 \pm 21.8$  s for newts in the UV-blocking treatment. This difference in time is not significant ( $t = -1.563$ ,  $df = 1$ ,  $p = 0.122$ ).

Newts in the UV-blocking treatment took  $362.6 \pm 33.1$  s ( $\bar{x} \pm$  SE) to leave the circle while newts in the UV-exposed treatment took only  $273.7 \pm 21.6$  s to leave the circle. There was a significant difference between treatments in time taken to leave the circle ( $t = -2.247$ ,  $p = 0.028$ ).

Newts in the UV-exposed and UV-blocking regimes both exhibited a significant orientation towards the water (as measured along the axis of the most direct route to the water) (UV-blocking treatment: Watson U<sub>2n</sub> = 1.053,  $n = 38$ ,  $p < 0.005$ , Fig. 2; UV-exposed treatment: Watson U<sub>2n</sub> = 0.950,  $n = 38$ ,  $p < 0.005$ , Fig. 2). There was no significant difference in orientation between these two treatments ( $U_2 = 0.086$ ,  $p > 0.20$ ).

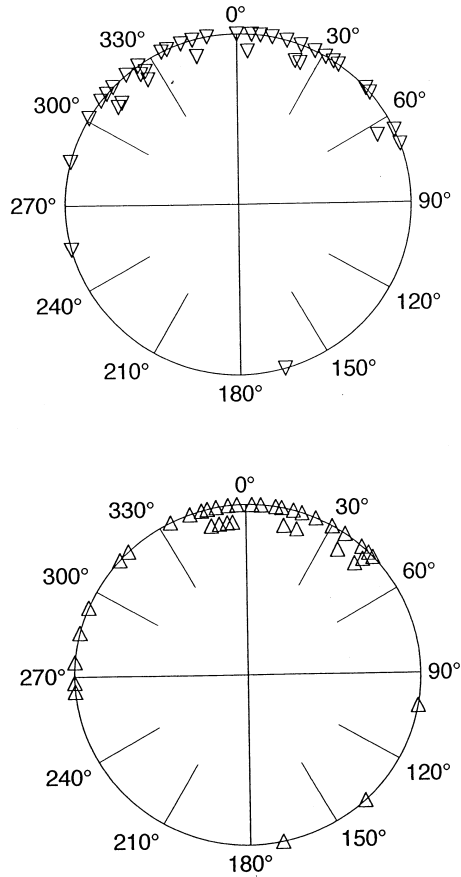


Fig. 2: Orientation of newts under UV-exposed (triangle; bottom panel) and UV-blocking (inverted triangle; top panel) treatments upon leaving the 6-m diameter test circle. (0 degrees corresponds with the axis of the most direct route to the water)

### Discussion

Our results show that exposure to relatively short-term but constant levels of UV-B radiation significantly affect at least one locomotor activity pattern in newts. We suggest that continued exposure to UV-B radiation was stressful to newts. The observed differences in locomotion between UV-exposed and control newts may be proximally related to neuroendocrine stress responses associated with the hypothalamo-pituitary-adrenal axis. In response to stressful conditions, corticotropin-releasing hormone (CRH) is secreted by hypothalamic neurons, which stimulates target neurons in the brain causing behavioral responses such as increased locomotion. For example, in both *T. granulosa* and laboratory rats, CRH stimulates locomotor activity during acute stress (Moore et al. 1984; Lowry & Moore 1991).

This is consistent with the increased locomotor behavior of UV-exposed newts in this study. Moreover, alpine newts (*Triturus alpestris*) exposed to simulated low levels of UV-B radiation exhibited erratic swimming activity in laboratory experiments (Nagl & Hofer 1997), which may also be a response to UV stress.

Several studies have shown that UV-B radiation is detrimental to amphibian embryos (e.g. Worrest & Kimeldorf 1976; Blaustein et al. 1994, 1998; Anzalone et al. 1998; Lizana & Pedraza 1998; Broomhall et al., in press), larvae (e.g. Worrest & Kimeldorf 1976; Nagl & Hofer 1997; Ovaska et al. 1997) and adults (Fite et al. 1998). However, few studies have investigated the sublethal effects of UV-B radiation. Even species whose eggs and larvae are unaffected by ambient levels of UV-B radiation may be affected at other life stages.

Seasonal increases in UV-B linked to ozone depletion over the Antarctic region are the best documented changes in UV-B radiation. (e.g. Frederick & Snell 1988; Solomon 1990) but statistically significant losses in total ozone have been observed in mid latitudes of both hemispheres as well (Frederick et al. 1989; Herman et al. 1996; Houghton et al. 1996). UV-B levels in relatively undisturbed alpine regions of the Swiss Alps have increased between 1981 and 1989 (Blumthaler & Ambach 1990). Kerr & McElroy (1993) have shown a 35% increase in winter and a 7% increase in spring in UV-B in Toronto, Canada, which they attributed to a downward trend in ozone (see also Herman et al. 1996). These reports suggest that UV-B flux may be increasing seasonally and that gradual expansion of the area of increased flux to lower altitudes and latitudes may be anticipated (Kerr & McElroy 1993; Madronich et al. 1998). We suggest that increased ultraviolet radiation may pose a significant stress to amphibians and perhaps to other wildlife.

Free-moving amphibians may be able to detect solar radiation and avoid large doses of UV-B in nature. However, over evolutionary time, selection pressure may have favored behaviors that exposed amphibians to prolonged bouts of UV-B radiation. Thus, in the Oregon Cascades, several species of frogs and salamanders lay their eggs in open shallow water, their tadpoles aggregate in open shallow water, and adults may bask in sunlight (e.g. Blaustein et al. 1994; Fite et al. 1998). Furthermore, under certain conditions, frog and salamander larvae cannot move out of sunlight, especially in open shallow ponds with little forest canopy (A. R. Blaustein, pers. obs).

Disturbances may exacerbate the effects of UV-B. For example, in southern California, periodic fires may influence how amphibians lay their eggs and find shelter (Gamradt & Kats 1997; Kerby & Kats 1997). Decreased canopy cover due to fire may temporally subject eggs, larvae and adults to increased doses of ambient UV-B, which in turn may hamper egg development, alter behavior and potentially adversely affect local populations (Kerby & Kats 1997). Similarly, clear-cutting in the Pacific North-west of North America has drastically decreased forest canopy cover (Norse 1990) where amphibians may find shelter (Walls et al. 1992). Unlike eastern forests that have been cut a little at a time over centuries, the north-western forests have been clearcut in large tracts, over decades (Ehrlich 1997). Thus, in a relatively short time, amphibians have been subjected to significant loss of habitat from which they otherwise could obtain shelter from solar radiation.

### Acknowledgements

We thank NSF (DEB-9423333) and the Katherine Bisbee Fund of the Oregon Community Foundation for support. We thank John Hays for use of the glass shields and spectroradiometer and valuable discussion, and Frank Moore for important discussions about newt behavior and physiology. We also thank Nareny Sengsavanh, Ramon Vargas, Susan Vargas, Hank Quinlan, and Pete Menzies for their help.

### Literature Cited

- Anzalone, C. R., Kats, L. B. & Gordon, M. S. 1998: Effects of solar UV-B radiation on embryonic development in three species of lower latitude and lower elevation amphibians. *Conserv. Biol.* **12**, 646—653.
- Batschelet, E. 1981: *Circular Statistics in Biology*. Academic Press, New York.
- Blaustein, A. R., Beatty, J. J., Olson, D. H. & Storm, R. M. 1995: *The Biology of Amphibians and Reptiles in Old Growth Forests in the Pacific Northwest*. USDA Forest Service General Technical Report PNW-GTR-337, Pacific Northwest Research Station, Portland, OR, pp. 1—98.
- Blaustein, A. R., Kiesecker, J. M., Chivers, D. P., Hokit, D. G., Marco, A., Belden L. K. & Hatch, A. 1998: Effects of ultraviolet radiation on amphibians: Field experiments. *Amer. Zool.* **38**, 799—812.
- Blaustein, A. R., Hoffman, P. D., Hokit D. G., Kiesecker, J. M., Walls, S. C. & Hays, J. B. 1994: UV-repair and resistance to solar UV-B in amphibian eggs: A link to amphibian declines? *Proc. Natl. Acad. Sci. USA.* **91**, 1791—1795.
- Blaustein, A. R., Kiesecker, J. M., Chivers, D. P. & Anthony, R. G. 1997: Ambient UV-B radiation causes deformities in amphibian embryos. *Proc. Natl. Acad. Sci. USA.* **94**, 13735—13737.
- Blumthaler, M. & Ambach, W. 1990: Indication of increasing solar ultraviolet-B radiation flux in alpine regions. *Science* **248**, 206—208.
- Broomhall, S., Osborne, W. & Cunningham, R. in press: Comparative effects of ambient ultraviolet-B (UV-B) radiation on two sympatric species of Australian frogs. *Conserv. Biol.*, in press.
- Duellman, W. E. & Trueb, L. 1986: *Biology of Amphibians*. McGraw-Hill, New York.
- Ehrlich, P. R. 1997: *A World of Wounds*. Ecology Institute, Oldendorf/Luhe, Germany. pp. 1—210.
- Fite, K. V., Blaustein, A. R., Bengston, L., & Hewitt, H. 1998: Evidence of retinal light damage in *Rana cascadae*: A declining amphibian species. *Copeia* **1998**, 906—914.
- Frederick, J. E. & Snell, H. E. 1988: Ultraviolet radiation levels during the Antarctic spring. *Science* **241**, 438—440.
- Frederick, J. E., Snell, H. E. & Haywood, E. K. 1989: Solar ultraviolet radiation at the earth's surface. *Photochem. Photobiol.* **50**, 443—450.
- Gamradt, S. C. & Kats, L. B. 1997: Impact of chaparral wildfire-induced sedimentation on oviposition of stream-breeding California newts, *Taricha torosa*. *Oecologia*. **110**, 456—549.
- Harte, J. & Hoffman, E. 1989: Possible effects of acidic deposition on a Rocky Mountain population of the tiger salamander *Ambystoma tigrinum*. *Conserv. Biol.* **3**, 149—158.
- Hays, J. B., Blaustein, A. R., Kiesecker, J. M., Hoffman, P. D. & Pandelova I., Coyle, C. & Richardson, T. 1996: Developmental responses of amphibians to solar and artificial UV-B sources: A comparative study. *Photochem. Photobiol.* **64**, 449—456.
- Herman, J. R., Bhartia, P. K., Ziemke, J., Ahmad, Z. & Larko, D. 1996: UV-B increases (1979–1992) from decreases in total ozone. *Geophys. Res. Letters* **23**, 2117—2120.
- Houghton, J. T., Meira, L. G., Filho, B. A., Callander, N., Harris, A., Kattenberg, A. & Maskell, K. (eds) 1996: *Climate Change 1995: The Science of Climate Change*. Cambridge Univ. Press, Cambridge.
- Kerby, J. L. & Kats, L. B. 1997: Modified interactions between salamander life stages caused by wildfire induced sedimentation. *Ecology*. **79**, 740—745.
- Kerr, J. B. & McElroy, C. J. 1993: Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science*. **262**, 1032—1034.
- Lizana, M. & Pedraza, E. M. 1998: Different mortality of toad embryos (*Bufo bufo* and *Bufo calamita*) caused by UV-B radiation in high mountain areas of the Spanish Central System. *Conserv. Biol.* **12**, 703—707.

- Lowry, C. A. & Moore, F. L. 1991: Corticotropin-releasing factor (CRF) antagonist suppresses stress-induced locomotor activity in an amphibian. *Horm. Behav.* **25**, 84–96.
- Madronich, S., McKenzie, R. L., Bjorn, L. O. & Caldwell, M. M. 1998: Changes in biologically active ultraviolet radiation reaching the earth's surface. In: *Environmental Effects of Ozone Depletion: 1998 Assessment* (van der Leun J. C., Tang, X. & Tevini M. eds). United Nations Environmental Programme, Nairobi, Kenya, pp. 1–27.
- Mason, R. T., Chivers, D. P., Mathis, A. & Blaustein, A. R. 1998: Bioassay methods for amphibians and reptiles. In: *Methods in Chemical Ecology: Bioassay Methods* (Haynes, K. F. & Millar, J. G., eds). Chapman & Hall, New York, pp. 271–325.
- Moore, F. L., Roberts, J. & Bevers, J. 1984: Corticotropin-releasing factor (CRF) stimulates locomotor activity in intact and hypophysectomized newts (Amphibia). *J. Exp. Zool.* **231**, 331–334.
- Nagl, A. M. & Hofer, R. 1997: Effects of ultraviolet radiation on early larval stages of the Alpine newt, *Triturus alpestris*, under natural and laboratory conditions. *Oecologia*. **110**, 514–519.
- Norse, E. A. 1990: *Ancient Forests of the Pacific Northwest*. Island Press, Washington, D.C.
- Nussbaum, R. A., Brodie, E. D. Jr & Storm, R. M. 1983: *Amphibians and Reptiles of the Pacific Northwest*. Univ. Press of Idaho, Moscow.
- Ovaska, K., Davis, T. M. & Flamarique, I. N. 1997: Hatching success and larval survival of the frogs *Hyla regilla* and *Rana aurora* under ambient and artificially enhanced conditions. *Can. J. Zool.* **75**, 1081–1088.
- Solomon, S. 1990: Progress towards a quantitative understanding of Antarctic ozone depletion. *Nature* **347**, 354.
- Tevini, M. (ed.) 1993: *UV-B Radiation and Ozone Depletion*. Lewis, Boca Raton.
- Walls, S. C., Blaustein, A. R. & Beatty, J. J. 1992: Amphibian biodiversity of the Pacific Northwest with special reference to old-growth stands. *Northwest. Environ. J.* **8**, 53–69.
- Worrest, R. C. & Kimeldorf, D. J. 1975: Photoreactivation of potentially lethal UV-induced damage to boreal toad (*Bufo boreas boreas*) tadpoles. *Life Sci.* **17**, 1545–1550.
- Worrest, R. C. & Kimeldorf, D. J. 1976: Distortions in amphibian development induced by ultraviolet-B enhancement (290–315 nm) of a simulated solar spectrum. *Photochem. Photobiol.* **24**, 377–382.

*Received: March 5, 1999*

*Initial acceptance: June 20, 1999*

*Final acceptance: August 9, 1999 (J.-G. Godin)*



Copyright of Ethology is the property of Wiley-Blackwell and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.