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Effect of UVA Radiation on Development and Hatching Success in *Oryzias latipes*, the Japanese Medaka

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Ultraviolet radiation (UVR), a component of the Sun's electromagnetic spectrum, is a ubiquitous feature of many of Earth's habitats. UVR is generally divided into three categories: UVA (320-400 nm); UVB (290-320 nm)U V C (200-290 nm). The precise wavelengths for each region may vary depending on the discipline involved and somewhat different points of division have been suggested (Diffey 1991). The intensity of light in each of these spectral subdivisions at the Earth's surface during daylight hours depends upon factors such as stratospheric ozone, solar altitude, and season. It is the first of these that is believed to effectively prevent wavelengths of less than about 290 nm from reaching the Earth's surface.

Diffey (1991) reviewed the organismal, cellular and molecular effects of wavelengths in the UVB region. Recent investigations have prompted concern for what appear to be declines in populations and/or ranges of some amphibian species. The possibility that increased levels of UVB may play a role in this process by affecting development has been discussed (Blaustein et al. 1994; Blaustein and Wake 1995; Blaustein et al. 1996 ; Hays et al. 1996; Kiesecker and Blaustein 1995) and debated (Licht 1996).

Studies of the effects of UVA radiation have documented both nuclear and anuclear cellular effects. DNA lesions, including single strand breaks, DNA-protein cross links, and pyrimidine dimer formation, have been observed in a variety of cell types (Peak et al. 1992; Sutherland et al. 1992). Oxidant stress and the generation of active oxygen intermediates are believed to play a pivotal role (Tyrrell 1992). Mitani (1996) has recently observed that UVA (and blue light) induces cyclobutane pyrimidine dimer photolyase activity in cultured goldfish cells. Non-nuclear effects in a variety of cells ranging from bacterial to mammalian include damage to membranes and membrane-bound

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organelles as well as lysis, nuclear-cytoplasmic separation and cytoskeletal damage (Godar and Beer 1992; Beer et al. 1993). Vile and Tyrell (1995) suggest that UVA damage to lipids and proteins in vitro or in human skin fibroblasts may involve iron, singlet oxygen and hydrogen peroxide as important redox species. UVA has also been shown to diminish the humoral immune response of splenocytes (Godar and Beer 1992) and to alter the immune functions of leucocytes (Leszczynski et al. 1996). At the organismal level Winckler and Fidhiany (1996) showed that exposure to UVA light produced a transient but significant decrease in metabolic rate of a Cichlid fish, Chiclasoma nigrofasciatum.

MATERIALS AND METHODS

In order to determine realistic ambient levels of near UVR, we made measurements of the irradiance of natural sunlight between 300 and 400 nm. Readings were taken in the vicinity of Princess Anne, Maryland at local apparent noon during the months of June, July, and August, 1996. These ranged from 420 $\mu w/cm^2$ on an overcast day to 2700 $\mu w/cm^2$ on a sunny day. All measurements of irradiance were made with a UVP J-221 longwave ultraviolet meter (UV Products, Upland, CA) with peak sensitivity at 365 nm.

Fertilized Medaka eggs were obtained from Carolina Biological Supply (Burlington, NC). Upon arrival each egg was placed in a separate compartment of a four-compartment acrylic Petri dish containing 5.5 ml. of an embryo rearing solution. At this time embryos were 24-36 hours old. Four dishes with four embryos each comprised the experimental group and a like number made up the control group. All dishes were kept in a walk-in environmental chamber whose temperature was maintained at 23° ± 0.5°C. The photoperiod regime was 14L 10D. All dishes were illuminated from below by two 40 watt cool white fluorescent bulbs. Experimental dishes were illuminated, in addition, by a UVA source which consisted of a bank of six Philips T-12 20 watt BLB lamps positioned 5 cm above the surface of the solution. Total spectral emission, based on manufacturer's specifications, was between 315 and 400 nm with only a minimal output below 350 nm and a peak emission at 365 nm. The irradiance at the water surface was 1450 $\mu w/cm^2$. Spectrophotometric studies indicated that the UVR absorbance of the solution overlying the embryos was minimal between 300 and 400 nm (.05 to .02). Thus, with approximately 2 mm of solution overlying the embryos, attenuation of UV irradiance was not a significant parameter.

All experimental and control dishes were covered with a commercial food wrap which prevented evaporation and was found to transmit 100 percent of the UVA radiation. Embryos were observed on a daily basis with a dissecting microscope allowing mortality as well as any gross developmental abnormalities to be noted. Cessation of heartbeat and circulation as well as accumulation of methylene blue dye by the embryo were used as end points for mortality. Embryos which hatched successfully were preserved in formalin for further study. A Chi-squared test (Barlow 1980) was used to determine the significance of differences in hatching success between experimental and control groups.

RESULTS AND DISCUSSION

Upon arrival all experimental and control organisms appeared to be developing normally and at stage five or six of Kirchen and West (1976). Figure 1 illustrates the cumulative daily mortality for the UVA exposed organisms. The data indicate that mortality occurred in "steps"; one following day two (stage 28) and a second on day eight (stage 34). No experimental animals survived beyond day nine. Two of the UVA exposed organisms hatched prematurely on days eight and nine. These individuals had not reached the level of development characteristic of control organisms prior to hatching. They lacked normally developed fins, did not show normal swimming movements, and exhibited a distinct angular malformation in the spinal column. For statistical purposes however, they were considered to have hatched. All control animals hatched between days 13 and 17. They appeared active and exhibited normal swimming movements. Statistical analysis indicated a highly significant difference ($P < 0.001$) between hatching success in experimental and control groups. Since all control group organisms developed normally and hatched we concluded that the plastic food wrap had no deleterious effects.

UVA appears to have a highly significant negative impact on hatching success in O. latipes. Although the combination of time and intensity of UVA exposure used in this study was relatively high, there are important insights to be gained from the results. Developmental events occurring at about days two and eight seem to be particularly vulnerable to perturbation by this type of radiation. It may be that morphological processes occurring at this time involve events that are especially susceptible to oxidant stress. This would be consistent with findings mentioned previously with respect to the disruption of basic cellular and molecular processes in a variety of cell types. Hyodo-

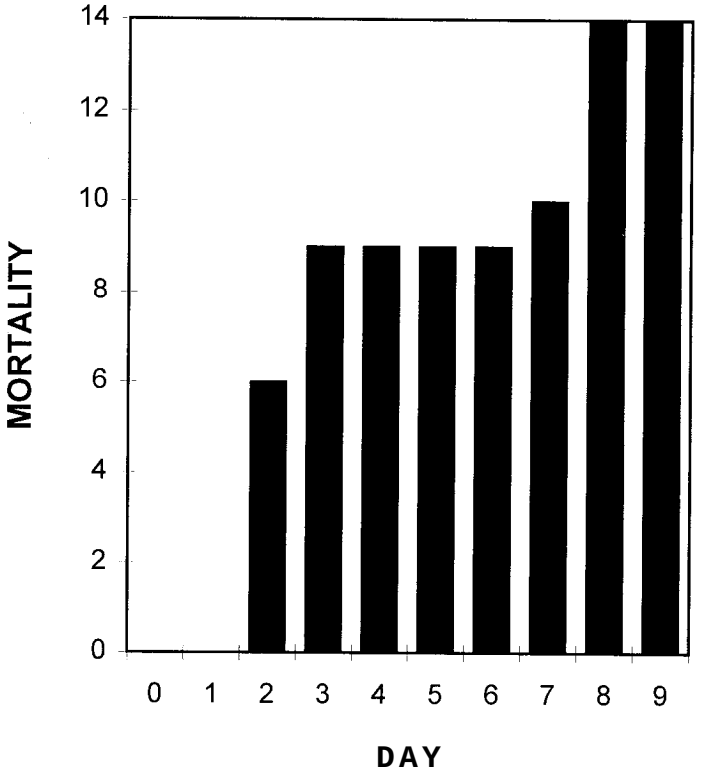

Figure 1. Cumulative daily mortality of UVA exposed medaka embryos. (n=16)

Taguchi (1983) found that a number of inbred strains of O. latipes, when irradiated at the morula stage, exhibited significant mortality at the time of optic bud formation as well as at hatching. It should be noted, however, that the radiation used in that study was in the "germacidal" region (typically 200 to 300 nm). Ijiri (1980) documented the high degree of sensitivity of newly fertilized eggs to UV radiation, but increased resistance to perturbations in development as a result of irradiation at later stages. Since all of the embryos used in our study were well beyond the zygote stage, it appears that developmental responses to UVA continue, although as noted, certain stages may be specifically more sensitive than others.

Ecologically, these results are important in illustrating the significance of UVA radiation to the development of organisms whose eggs are deposited in shallow, open water habitats. If the putative negative effects of UVB and UVA radiation act synergistically

they could affect developmental processes and thereby exert significant pressures on these populations. Additionally, in field studies conducted under conditions involving natural sunlight one cannot afford to ignore the presence of UVA and its effects when UVB is the radiation of primary interest.

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