THE KINETICS OF AVOIDANCE OF SIMULATED SOLAR UV RADIATION BY TWO ARTHROPODS

JEANNE A. BARCELO AND JOHN CALKINS, *Department of Radiation Medicine and School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40536 U.S.A.*

ABSTRACT There is an increasing likelihood that the solar UV-B radiation ($\lambda = 280–320$ nm) reaching the earth's surface will increase due to depletion of the stratospheric ozone layer. It is recognized that many organisms are insufficiently resistant to solar UV-B to withstand full summer sunlight and thus mechanisms which facilitate avoidance of solar UV-B exposure may have significance for the survival of sensitive species. There are many alternative pathways which would lead to avoidance of solar UV-B. We have investigated the dynamics of biological reactions to simulated solar UV-B radiation in two small arthropods, the two-spotted spider mite *Tetranychus urticae* Koch and the aquatic copepod *Cyclops serrulatus*. Observations of positioning and rate of movement were made; a mathematical formalism was developed which assisted in interpretation of the observations. Our observations suggest that, although avoidance would mitigate increased solar UV-B effects, even organisms which specifically reduce their UV-B exposure would encounter additional stress if ozone depletion does occur.

INTRODUCTION

The measurement of the tolerance of a wide variety of organisms indicates that most species, even those clearly requiring sunlight, such as algae, must limit their sunlight exposure to levels less than the amount reaching the earth's surface in temperate or tropical summer (1–3). Since many common species could be killed or severely injured by amounts of solar UV-B (wavelength 280–320 nm) exposure which they potentially might receive on a sunny summer day, it is evident that mechanisms to avoid or limit exposure to solar UV would be biologically advantageous. Some organisms are able to avoid sunlight either by behavior or choice of habitat. However, organisms adapted to total darkness are rare and the familiar biota evidently compromise between the advantages of living in the photosphere and the injuries inflicted by sunlight. Higher plants, since they occupy fixed positions, are obligated to have a UV resistance adequate to tolerate the solar radiation incident upon them, but the biota which can change location (animals, planktonic algae, etc.) have a potential to adjust solar UV exposure in the most advantageous way (4–6). It has been shown that UV-B itself induces movement in a wide variety of organisms (6, 7). We propose a model relating positioning behavior to UV-B exposure which may elucidate techniques motile organisms might utilize to respond to solar UV exposure. The behavior predicted from this model will be compared with the observed UV-B positioning responses of two arthropods.

BIOPHYS. J. © Biophysical Society · 0006–3495/80/12/921/09 $1.00
Volume 32 December 1980 921–930

MATERIALS AND METHODS

Test Organisms and Culture

Tetranychus urticae Koch, the two-spotted spider mite, was kindly supplied by Dr. Juan G. Rodriguez of the University of Kentucky, Department of Entomology. Mites were grown on bean leaves maintained under constant illumination. Test mites were discarded after each experiment. *Cyclops serrulatus,* an aquatic copepod, was isolated from local waters in 1976 and grown on an algae-protozoan medium. Only adult animals were tested; all growth and tests were performed at ambient room temperature (24° ± 3°C).

Mite Test Apparatus

To examine the ability of *T. urticae* to detect and avoid the various intensities of UV-B irradiation, mites were placed on strips of diagonally cut bean leaves ~6.5 cm long and 1.5 cm wide. The test area was encircled with a sticky resin (tanglefoot) which confines the animals to the desired section of the leaves. The leaf sections were kept moist and cooled by floating in water in 100-mm petri plates during tests. Half of each leaf test area was exposed to UV-B wavelengths and the other half to UV-A and visible light; the boundary was across the short dimension of the rectangular leaf section. A lucite filter (which transmits wavelengths longer than 350 nm [6]) was used to eliminate the UV-B wavelengths on the shielded side of the leaf. To equalize the total irradiance (measured using a thin film quartz window thermopile) on both sides of the leaf, a wire screen was placed over the UV-B side to act as a neutral density filter, transmitting the UV-B but cutting the total irradiance to equal the total irradiance of the lucite filtered side.

Twenty mites were loaded onto the UV-B exposed end of the leaf; in reverse experiments, all 20 mites were loaded onto the shielded side of the leaf. The leaf loaded with the mites was then placed into the irradiator. At periodic intervals the distribution of the mites on the leaf was observed.

Copepod Test Apparatus

Cyclops swims rapidly and can locate in one of two connected chambers cut in a lucite block (6). The optical filter system used for this apparatus was identical with the mite assay system, i.e., one chamber was exposed to the UV-B through a neutral density filter (screen) covering the UV chamber and the other with a lucite filter that eliminated wavelengths below 350 nm. Both chambers received equal total irradiance, the radiation varying only in spectral composition.

Fifteen test organisms were loaded into the UV chamber with the "gate" (teflonplug) between the chambers closed. The gate was then opened as the system was placed under illumination. At predetermined times the gate was closed and the number of organisms in each chamber counted. After determining their distribution, the gate was reopened, allowing the animals to again swim freely between chambers for subsequent counts.

Radiation System

As in previous experiments, the "simulated solar UV-B" was produced using Westinghouse FS-20 lamps (Westinghouse Electric Corp., Pittsburgh, Pa.) and a filter of ⅛" pyrex glass (6, 8). The spectral composition of the simulated solar UV-B approximated sunlight with the ozone column of 0.16 cm O_3. The system was calibrated using the Robertson sensor (see Berger [9] for a discussion of the sunburn unit [SU]); in our system 1.0 SU/h corresponds to 2.8 $J/m^2/s$ of total radiation.

Independent Determination of Rate of Movement

Various parameters were microscopically observed which relate to the rate of movement of the two animals upon various levels of exposure to radiant energy. The average pause between swimming movements of *Cyclops* was determined by direct observation; also the average time to swim from one test chamber to another when both chambers were equally illuminated was observed. The average movement of mites along a narrow leaf strip was determined by direct observations repeated at ∼5-mi intervals.

RESULTS

Responses of Mites

The redistribution of animals between exposed and shielded areas followed a systematic and reproducible pattern. There was no "gate" to restrain the mites and confine them to the desired half of the test area while they recovered from the disturbance involved in loading the 20 animals onto the test leaf. During the initial period of 20 min an average of 25–35% of the animals wandered out of the starting area with the net loss of population essentially unrelated to the UV-B exposure. The behavior for the remaining test period was clearly related to UV-B exposure rate. Fig. 1 illustrates the time course of distribution of mites. Each point was the

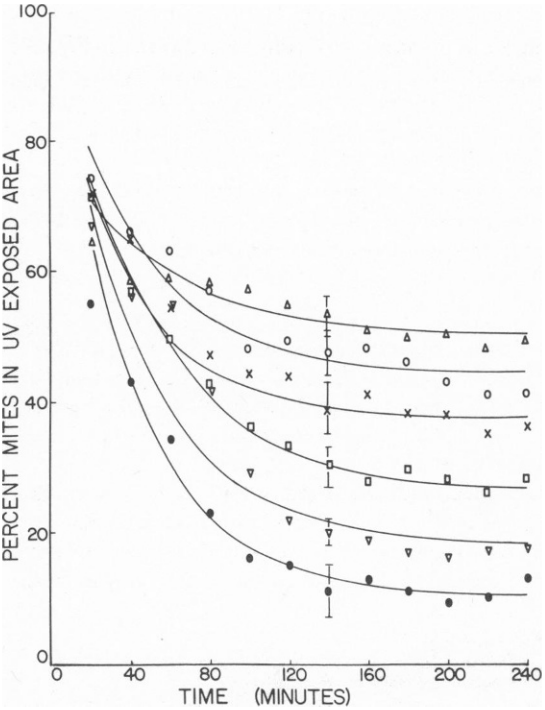

FIGURE 1

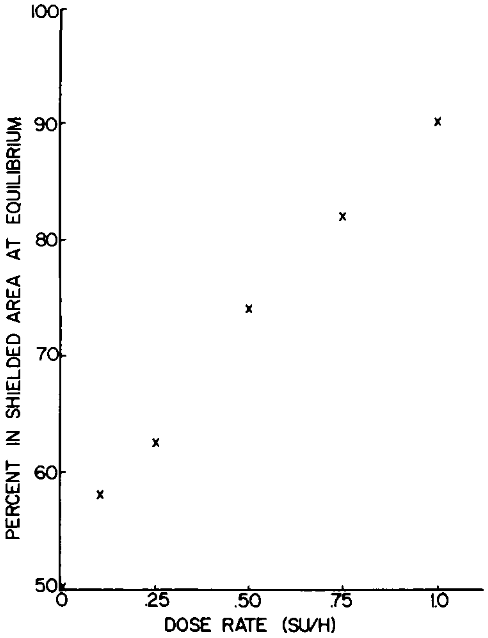

FIGURE 2

FIGURE 1 The dose rate dependence and time course of redistribution of the mites *T. urticae* Koch to simulated solar UV-B. Exposures were begun at $t = 0$; dose rates: △ = 0 SU/h, 0 = 0.1 SU/h, X = 0.25 SU/h, □ = 0.5 SU/h, ▽ = 0.75 SU/h, ● = 1.0 SU/h. Each point was the mean of at least 24 independent determinations. The lines were fitted using Eq. 4; bars indicate the SEM of all 140-min points and are typical of the SEM of the other points which were omitted for clarity of presentation.

FIGURE 2 The dose dependence of the distribution of mites where equilibrium is attained. With increasing dose rates the mites are more restricted to the shielded portion of the system. The response appears to be essentially linear with dose.

average determined from 24 independent tests. Two phases are evident in Fig. 1: first, a transition phase lasting for ~120 min, followed by a dose-rate-dependent equilibrium phase where the distribution is constant with time. In the absence of stimulus, mites attain an equal distribution in the two areas; however, increasing UV-B dose rate progressively compresses the mites into the protected area. The relationship of dose rate to the deviation from equal distribution is illustrated in Fig. 2, the deviation being essentially linear with dose rate over the range tested.

Equilibrium can be considered a balance between two "forces": "the diffusive force" of the excess population balanced by the "UV avoidance force." The attainment of equilibrium would be expected to progress at a rate dependent on the imbalance of these two forces. The equilibrium distribution of animals should be attained irrespective of the starting distribution; the equilibrium values observed are equal and independent of starting conditions whether the animals were started all in UV or all in the shielded areas.

Cyclops Results

The time course of positioning behavior of the aquatic copepod *Cyclops* is shown in Fig. 3. *Cyclops* also shows a specific avoidance of the UV-B exposed area and attainment of an

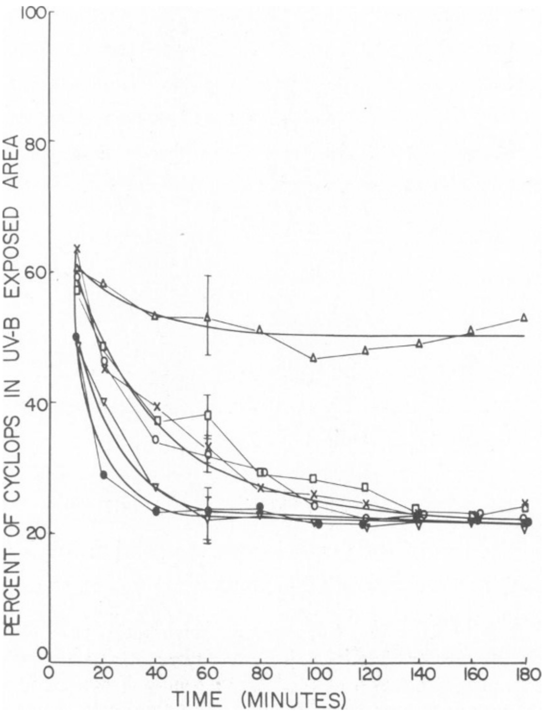

FIGURE 3 The dose rate dependence and time course of redistribution of the aquatic copepod *C. serrulatus* upon exposure to UV-B. Exposures were begun at $t = 0$; dose rates: △ = 0 SU/h, 0 = 0.1 SU/h, *X* = 0.25 SU/h, □ = 0.5 SU/hr, ▽ = 0.75 SU/h, ● = 1.0 SU/h. The data points were fitted using Eq. 4; curves fitting the 0.25 and 0.50 data points were omitted since they were essentially coincident with the curve fitting the 0.10 data point.

equilibrium distribution; however, the detailed patterns of response differ from the mite responses. There is an early dose-independent redistribution when the gates are opened leading to roughly equal numbers of animals in both compartments 10 min after the exposure begins. After the initial (10 min) transient period, the further attainment of equilibrium is a complex dose-dependent function (see Discussion section). The attainment of equilibrium is very rapid (half time, ~6 min) after the highest dose, falling to 26 min for the three lowest doses (Fig. 6). Attainment of equilibrium with animals loaded in the shielded chamber was tested at the highest irradiance (data not shown). The same pattern was observed as when the animals were loaded in the UV-B chamber (i.e., approximately equal distribution 10 min after opening the gate, after which the animals moved into the equilibrium distribution of 22% with a time course similar to the other loading procedure).

DISCUSSION

For analytical purposes we do not assume the subject organisms possess capacities to survey and retain in memory a map of their environment remote from their physical bodies. We assume directed movements originate from immediate stimuli only; i.e., that it is possible for even a simple animal to recognize that (*a*) UV-B is incident or that (*b*) upon a given movement UV-B is either constant, increasing, or decreasing. If UV-B accelerates movement or if movements which increase the UV-B exposure are reversed (4) while those decreasing UV-B exposure are continued, the organisms will eventually collect in protected areas. The removal of animals from an exposed area when they are accelerated in random motion by UV or inhibited from returning to a UV exposed area will be a random process dependent on the numbers present. The rate of removal from the UV exposed area under the condition that the animals will not return to the exposed area can be expressed by the common differential equation:

$$-\frac{dN}{dt} = KN, \tag{1}$$

where N is the number of animals in the exposed area, t is time, and K is dose-rate dependent constant related to the rate of random movement of organisms and the geometry of the exposed and unexposed areas. The solution of Eq. 1:

$$N = N_0 e^{-Kt}, \tag{2}$$

would describe the time course of the decline in numbers of animals in the exposed chamber (assuming no return), N_0 being the number of animals in the exposed area at $t = 0$.

A more realistic model, which fits our observations, can be generated by assuming that the barrier to the return to the exposure area is "leaky" and that a fraction of the shielded animals will wander back into the exposed area, R, being the rate constant for return to the UV area. The leakiness of the barrier (R) could depend on various factors, such as the gradient of UV-B dose rate or the rate of movement in the exposed and protected areas. We remind the reader that the irradiance in the exposed and protected areas was always equal; when UV-B dose rate increases there would be an increase in longer wavelengths in the shielded area which could affect the constant R analogous to the effects of UV-B on the constant K. The time dependence of motion can be determined by integrating the differential equation:

$$-\frac{dN}{dt} = KN - R(N_0 - N), \tag{3}$$

where the symbols have the meaning previously defined.

The integrated form of Eq. 3 with the boundary conditions set by the biological problem is given as Eq. 4:

$$N = N_0 \frac{R}{R + K} + N_0 \frac{K}{R + K} e^{-(R + K)t}. \tag{4}$$

Eq. 4 reduces to Eq. 2 when $R = 0$; when $t \rightarrow \infty$ Eq. 4 reduces to Eq. 5:

$$\frac{N}{N_0} = \frac{R}{R + K}, \tag{5}$$

which provides an easy way to evaluate the constants K and R. Without stimulus, $K = R$. The value of the sum of R and K determined the slope of the transient response and the data plotted in Figs. 4 and 6 can be used to compute $R + K$ for the various dose levels. The observed equilibrium distributions (N/N_0) combined with values of $R + K$ then define both R and K (Eq. 5).

Figs. 5 and 7 show the dose dependence of R and K computed from the positioning observations (data presented in Figs. 1 and 3).

While the positioning observations establish the dose dependence of K and R, there are many possibilities as to how the observed variations of K and R could arise. A dose dependent UV-B increased rate of movement could be postulated and would increase K; it seems less likely that R would be reduced by irradiation with light lacking UV-B. The hypothesis that the variations in K and R arise from rate of movement can be tested in both animals. Figs. 5 and 7 show the results of such tests. Although the mites are accelerated by UV-B exposure (Fig. 5), the increased movements alone do not seem to provide an adequate explanation of dose dependences of K and R. In the case of *Cyclops* (Fig. 7) the observed rates of movements under the various radiations seem generally to confirm the variations of K and R as determined by positioning behavior illustrated in Fig. 3. We also observed in *Cyclops* that UV-B exposure tended to slow subsequent movement when the UV-B component was removed.

Our observations show that organisms react specifically to the short wavelength components of solar radiation. The outcome of these reactions would, under natural conditions, reduce the animal's exposure to UV-B and thus these animals have some capacity to reduce the impact of potential increases in solar UV-B in the event of reduction of the ozone layer. If there is mixing of populations, i.e., individual animals frequently exchanging between the exposed and protected areas, then the average exposure in the mite population (within the dose rate range we have tested) will approximate 0.15 SU/h at all dose rates > 0.25 SU/h. Ozone depletion will increase the length of time on any given day when solar UV-B exceeds 0.25 SU/h and thus the average daily exposure of mites would increase with ozone depletion. The behavior of Cyclops is also such that the average exposure of a population would increase

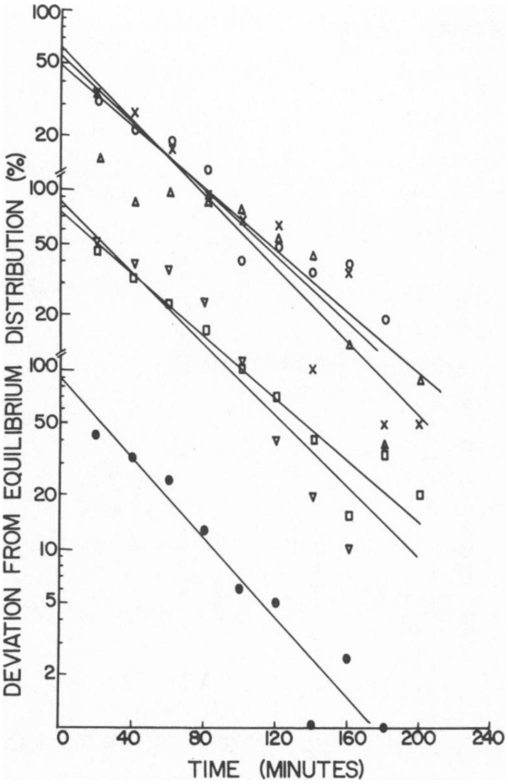

FIGURE 4

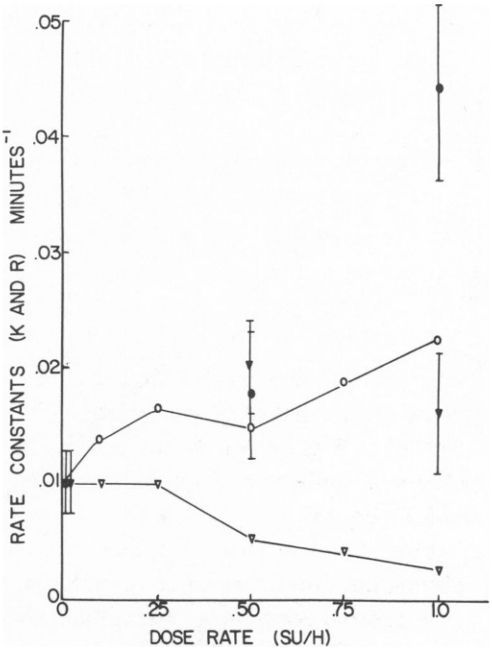

FIGURE 5

FIGURE 4 The time course of attainment of equilibrium of mites upon exposure to various UV-B dose rates. The deviation was determined by subtracting the equilibrium value (%) from the observed portion of population in the exposed area at various times. An exponential attainment of equilibrium is evident. Different dose rate data was separated for clarity of presentation: △ – 0 SU/h, ○ – 0.10 SU/h, X – 0.25 SU/h, □ – 0.5 SU/h, ▽ – 0.75 SU/h, ● – 1.0 SU/h.

FIGURE 5 The dose rate dependence of the constants *K* (○) and *R* (▽), connected points, deduced from the positioning behavior of mites (data in Fig. 1) and from direct observations of movements of single mites along narrow strips of leaves under various levels of exposure to UV-B and filtered (UV-B removed by lucite) radiation of equal irradiance (isolated points, ▼ – *R*, ● – *K*, with bars indicating the SEM of the determinations). The ○ dose point was determined using orange light of irradiance less than the 1.0 SU irradiance (2.8 $J/m^2/s$).

even more than is the case for the mite population since the equilibrium distribution did not progressively decrease with increasing dose rate. Our observations suggest that populations of organisms which specifically sense and avoid UV-B would still receive increased UV-B upon ozone depletion, although not in direct proportion to the increase of incident UV-B energy at the earth's surface.

A second point of concern regarding the ecological ramifications of reduction of the ozone layer relates to the exact wavelength which stimulates the avoidance movements in these animals. Our tests demonstrate that avoidance is stimulated by wavelengths shorter than

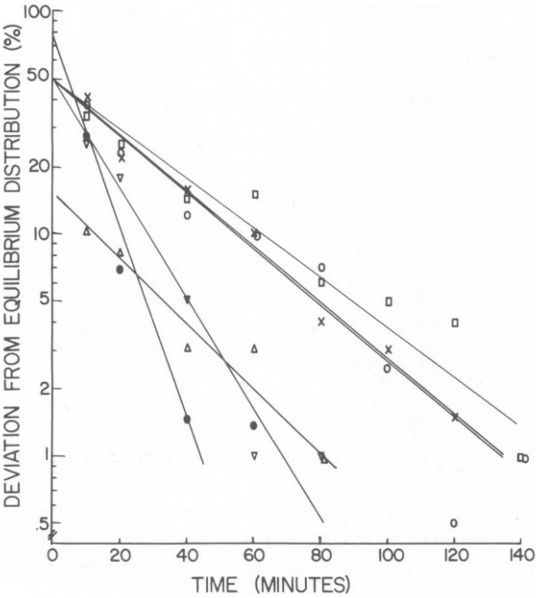

FIGURE 6

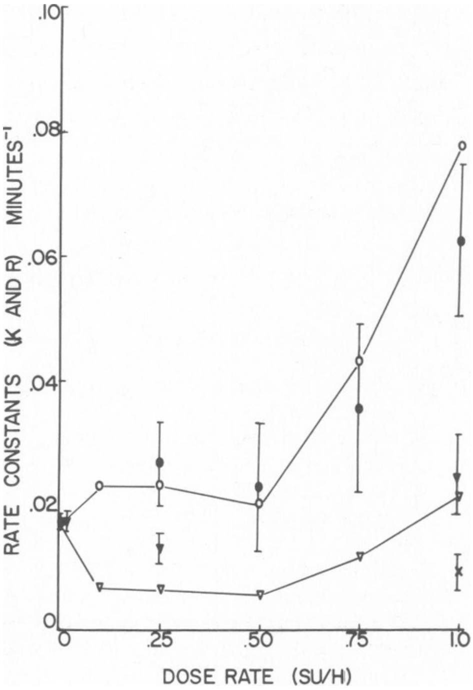

FIGURE 7

FIGURE 6 The dose rate dependence of time course of attainment of equilibrium of *Cyclops* plotted as in Fig. 4. Dose rates of: 0.1 SU/h (O), 0.25 SU/h (x), and 0.5 SU/h (□) showed similar slopes. The higher dose rates 0.75 SU/h (▽) and 1.0 SU/h (●) showed more rapid attainment of equilibrium. The response without exposure (△) reflects the initial disturbance leading to resorting of animals with only a 10% excess of animals in the chamber containing all the animals at $t = 0$ after only 10 min. Following this initial redistribution, equilibrium is attained at the same rate observed after low UV-B exposure rates.

FIGURE 7 The dose rate dependence of the constants K (O) and R (△) deduced from the positioning observations of *Cyclops* plotted in Fig. 3 (connected points). The isolated points (● – K, ▼ – R) were values of K and R deduced from observations of the average rate of movement of *Cyclops* exposed to the various light sources and intensities. A single *Cyclops* was located in a two chamber system. The animals moved erratically about in the chamber and would from time to time switch from chamber to chamber. The two chambers were illuminated with either UV-B, UV-B filtered light of equal irradiance, or very dim orange light. The number of switches from chamber to chamber per unit time is assumed to represent the net rate of movement of the animal under the various radiations. From the net rate of movement the constants K and R were independently determined. The O dose rate point was determined observing the animals with orange light of total irradiance less than the 1.0 SU irradiance. The variation of R suggests that the animals move at a slower rate with filtered light (near UV-blue), than with no light or orange light. The direct observations seemed to verify this conclusion. In all cases UV-B accelerated the rate of movement. It was observed (x) that pre-exposure to 1.0 SU UV-B greatly slowed the subsequent rate of movement in the filtered light. The observations presented in this figure are consistent with a UV-B avoidance mechanism based entirely on acceleration or deceleration of rate of movement dependent only on the nature of incident radiation and its dose rate.

~350 nm. Because of the ozone absorption spectra, depletion of the ozone layer would greatly increase solar UV-B, but would have little effect on wavelengths longer than ~330 nm. If the avoidance reactions are primarily stimulated by wavelengths <330 nm, then the animals would compensate, in part, for increased exposure. It is, however, possible that the stimulus for avoidance arises from absorption of energy between 330 and 350 nm (5) and some organisms which avoid UV-B exposure would not receive a stronger avoidance signal even though the UV-B component of sunlight were greatly increased.

The work on which this report was based was supported in part by the Office of Water Research and Technology, U.S. Department of Interior, under the provisions of Public Law 88-379.

Received for publication 24 January 1980 and in revised form 18 June 1980.

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