Photochemistry and Photobiology, 2000, 72(3): 314–319

Biological Weighting Function for the Mortality of *Boeckella gracilipes* (Copepoda, Crustacea) Derived from Experiments with Natural Solar Radiation¶

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Received 26 January 2000; accepted 8 June 2000

ABSTRACT

We performed *in situ* experiments during the austral summer of 1998 to quantify the mortality of the freshwater copepod *Boeckella gracilipes* as a function of the UV dose. The copepods were exposed to solar radiation at the water-surface for ~24–34 h. Long-pass cut-off filters (Schott) were used in the exposure experiments. UV radiation and PAR were measured with an IL-1700 (International Light Inc.) and a PUV-500 radiometer (Biospherical Instruments Inc.). A biological weighting function for UV-induced mortality was calculated by fitting a model based on a logistic curve. Our results show that UV damage in this species is strongly wavelength- and dose-dependent. *B. gracilipes* was highly vulnerable to both UV-B (290–320 nm) and UV-A radiation (< 360 nm). The shape of the BWF obtained for *B. gracilipes* resembles more closely the action spectra (AS)† for UV-induced erythema, than the AS for naked DNA.

INTRODUCTION

To evaluate the biological effects of solar UV radiation (290–400 nm) on aquatic organisms, it is necessary to quantify measured responses, for example mortality, as functions of irradiance and time. Because UV-induced effects are strongly wavelength-dependent (1), biological weighting functions (BWFs) must be determined to relate those responses quantitatively to UV exposure. BWFs are determined by exposure of e.g. DNA, membranes, cells, whole organisms to polychromatic radiation at different wavelengths (2,3). Weighted irradiance spectra show which wavelengths are most biologically effective, regardless of the shape of the irradiance spectrum (4).

Wavelengths less than 320 nm can result in significant biological damage due to their high energy content per photon (5). UV damage can be produced by three photochemical processes: (i) The absorption of nucleic acids can directly result in DNA and RNA destruction (mainly caused by UV-C [< 290 nm] radiation). The effects depend on the absorption characteristics of the chromophores. For example, UV-induced DNA damage, measured as cyclobutane pyrimidine dimers, is shown in Antarctic zooplankton during periods of high UV-B radiation (6). (ii) Effects may be caused by protein absorption that can indirectly lead to intermediate excited compounds producing reactive species (mainly caused by UV-B radiation). Several reactive oxygen species (ROS) are produced in the presence of UV radiation (7). The formation of radicals can damage biomolecules and cells (8), and can result in erythema and indirect melanization. (iii) UV-absorption can lead to a shift in the redox equilibrium resulting in direct melanization without erythema (mainly caused by UV-A radiation). In general, effects caused by UV-B (290–320 nm) radiation are about three times higher than those caused by UV-A (320–400 nm) radiation (9).

Several freshwater zooplankton species are negatively affected by current levels of UV-B radiation (10,11). However, the response to UV radiation shows variability among species (12–18), life-stages (11), pigmented *versus* unpigmented morphs (19) and even within one single species (16,20,21). Moreover, reduced survival and fecundity in copepod (*Diaptomus*) females have been reported (14). Depending on the morphology of an organism, and on the presence or absence of photoprotective pigments, different BWFs may be expected. Freshwater copepods differ in their UV responses, although they are generally small, and photoprotective compounds (e.g. carotenoids, UV-absorbing compounds) are commonly found in those organisms. For example, different carotenoid concentrations are shown among different copepod species (21), life-stages (22), vertical distribution (23) and habitat (24). Furthermore, the presence or absence of photorepair mechanisms, like photoenzymatic DNA repair, can influence UV-induced impairment. However, photoreactivation is observed in several zooplankton taxa (10,12,13,16), while not in others (16,19).

Most commonly, effects of UV radiation on zooplankton have been quantified as general functions or as functions of broad-band measurements (*i.e.* UV-B and/or UV-A), where ranges were considered separately by using UV-B cut-off

¶Posted on the website on 21 July 2000.

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†*Abbrevations:* AS, action spectra; BWF, biological weighting function; DOC, dissolved organic carbon; PAR, photosynthetically active radiation; ROS, reactive oxygen species.

filters such as Mylar D (14–17,20,21). To our knowledge, there is only one study that gives information on the effects of artificial UV radiation on a marine copepod using long-pass cut-off filters (25).

Here we present results from *in situ* experiments designed to derive BWF for the mortality of the calanoid freshwater copepod *Boeckella gracilipes* under natural solar radiation. We choose this species because there is evidence that UV-induced mortality in *B. gracilipes* obeys the principle of reciprocity, which allows the combination of data obtained from different UV exposure experiments.

Materials and Methods

Zooplankton, site and sampling. Experiments to assess the effect of solar UV-B radiation on the survival of *B. gracilipes* Daday were performed during January and February 1998 (austral summer). The translucent calanoid copepod *B. gracilipes* is typically living in deep, ultra-oligotrophic lakes (where it usually stays deep during daytime), but also occurs in shallower, more productive aquatic systems (H. Zagarese, B. Tartarotti, personal observation). Zooplankton samples were taken with a net (55 μm mesh size) by making multiple horizontal tows from Lake Trébol (41°03′S, 71°31′W; Northwestern Patagonia, Argentina). This lake is slightly turbid and oligotrophic, with a mean concentration of 1.7 g m^{-3} dissolved organic carbon (DOC), a 10% attenuation depth (305nm) of ~0.5 m, and a mean chlorophyll *a* concentration of 0.9 μg l^{-1}. The day before the experiments started, 25 copepodids C V (*i.e.* the last juvenile stage before molting into adults) and adult individuals of *B. gracilipes* were transferred into glass flasks and kept at 10°C (dark). The percentage of copepodid and adult individuals was roughly constant in the different exposure experiments.

Experimental design. The copepods were placed in glass dishes (KIMAX, 60 mm × 35 mm) filled with screened (20 μm mesh size) lake water. The wall of the dishes was covered with black tape. Five dishes, each containing 25 copepods, were placed on top of a black tile; the upper rim of the dishes was covered with a polyethylene band to avoid evaporation. Quartz substrate long-pass Schott filters (Andover Corporation, Salem, NH; 165 mm × 165 mm) were fixed onto the dishes and the tile. Filters with different cut-off wavelengths for UV and photosynthetically active radiation (PAR, 400–700 nm) were used. Each treatment was run in five replicates. The treatments were incubated in a black fish raceway (50 cm × 150 cm) with constant water flow in order to maintain the temperature constant (~16°C).

Experiments. Three independent experiments were run for ~24 to 34 h. In the first experiment (8–9 January 1998), four treatments with different cut-off filters for UV (Schott WG-305, WG-320, WG-345, GG-375) and one PAR-treatment (Schott GG-395) were used. One additional treatment served as dark control (filter glass wrapped with two layers of aluminum foil) to have an estimate of the background mortality in the absence of solar radiation. The dishes wrapped with aluminum foil were placed away from the remaining dishes to avoid potential reflection. The second experiment (17–18 January 1998) consisted of six UV-treatments (Schott WG-295, WG-305, WG-320, WG-335, WG-360, GG-375), two PAR-treatments (Schott GG-395, GG-420) and a dark control. For the third experiment (February 4 to 5 1998), five UV-treatments (Schott WG-295, WG-305, WG-320, WG-335, WG-360) plus a dark control were used.

In the laboratory, the copepods were counted under a dissecting microscope (10 × 16 magnification) immediately after the end of the experiment. The number of dead and live organisms was recorded.

Radiation measurements, calculations of dose and BWFs. Ultraviolet and PAR irradiances were measured with an IL-1700 portable radiometer from International Light Inc. During the second experiment, a portable PUV-500 multichannel filter radiometer (Biospherical Instruments Inc.) was additionally used for data comparison. The former radiometer is equipped with UV-B and UV-A wide-band detectors centered at 295 and 356 nm, respectively; and a PAR detector with a flat response between 400 and 700 nm. The sensors of the PUV-radiometer have bandwidths ≤10 nm for the UV channels and measure at the following nominal wavelengths: 305, 320, 340 and 380 nm plus PAR (for a description of the instruments, see Kirk *et al.* [26]). Although complete spectral, high-resolution data cannot be obtained from these instruments, they do compare conveniently with some spectroradiometers of higher resolution (26,27). Ground level irradiances were recorded every 5–15 min during the experiments. Independently, we calculated spectral irradiances using a simplified radiative transfer two-stream code (see for example Frederick and Lubin [28]). Stratospheric ozone concentration was derived from satellite data (TOMS) for the region of Bariloche and the experimental days. Cloud optical depth was left as a fitting parameter. Its best value was found by comparing UV-A and UV-B irradiances derived from the calculated spectrum with actual measurements performed with the broadband radiometers. Once the spectral irradiance $I(\lambda)$ was determined, we calculated the integrated irradiance for each band D_i, by convoluting the spectral irradiance with the transmission curve of each filter used in the experiment $F_i(\lambda)$:

$$D_i = \int I(\lambda) \cdot F_i(\lambda)\, d\lambda \qquad (1)$$

To relate the observed mortality with the UV dose (*i.e.* accumulated irradiance received by the copepods) for each treatment, we used a model, already proposed for this species in a previous paper (29). The model is based on the logistic curve:

$$d = \frac{\exp(k + a \cdot D)}{1 + \exp(k + a \cdot D)} \qquad (2)$$

where d is the proportion of dead individuals, k a measure of the condition of the organisms (note that $\exp(k)/(1+\exp(k))$ is the mortality in the control treatment), a a measure of the sensitivity of the organism to the UV dose D. In our case the model used was extended to include "spectral sensitivity", *i.e.* a BWF for the mortality

$$d = \frac{\exp(k + a_1 \cdot D_1 + a_2 \cdot D_2 + \cdots)}{1 + \exp(k + a_1 \cdot D_1 + a_2 \cdot D_2 + \cdots)} \qquad (3)$$

D_i is the accumulated dose for the ith band, and a_i the biological weight for that band. We choose a_i units in such a way that $a_i D_i$ is dimensionless. We fitted the model using the Systat NONLIN procedure.

Results

Mortality

In the first experiment, the mean mortality of *B. gracilipes* in the dark controls was 33.2% (8.3 individuals). The mean mortality in the control (PAR 395 nm) was 39.0% (9.8 individuals). In the UV-treatment groups, mean mortality ranged between 30.1 (7.5 individuals) and 96.7% (24.2 individuals) (Table 1). The data of the second experiment showed low mean mortality in the dark control (6.6%, or 1.7 individuals), the PAR and the 385, 375, 360 nm UV-treatment groups (5.4–22.9%, or 1.4–5.7 individuals). In the 335 nm treatment, the mean mortality was 76.8% (19.2 individuals), while in the UV-B treatment groups it was >88% (>22 individuals) (Table 1). In the third experiment, the mean mortality in the dark controls was 3.6% (0.9 individuals). Mean mortality was low (11.7%, or 2.9 individuals) in the 360 nm treatment, while in the other UV-treatment groups it ranged from 16.0 to 65.4% (4–16.4 individuals) (Table 1).

Dose

The spectral doses in the UV range obtained during the experiments are given in Fig. 1. The first two exposure-exper-

Table 1. Dose (D), cumulative dose (D_{cum}) and mean mortality (M, ± 1 standard deviation (s.d.)) of *Boeckella gracilipes* experienced during the three independent UV exposure experiments

Waveband (nm)	1st Exp. (8–9 January 1998)			2nd Exp. (17–18 January 1998)			3rd Exp. (4–5 February 1998)		
	D (J cm^{-2})	D_{cum} (J cm^{-2})	M (%) (s.d.)	D (J cm^{-2})	D_{cum} (J cm^{-2})	M (%) (s.d.)	D (J cm^{-2})	D_{cum} (J cm^{-2})	M (%) (s.d.)
Dark control	0	0	33.15 (16.62)	0	0	6.62 (6.65)	0	0	3.64 (5.93)
420–700	–	–	–	26.8	26.8	5.42 (4.24)	–	–	–
395–400	28.90	28.90	38.97 (21.42)	–	–	–	–	–	–
395–420	–	–	–	130.0	156.8	8.62 (6.49)	–	–	–
375–395	129.12	158.02	30.14 (10.00)	–	–	–	–	–	–
360–400	–	–	–	–	–	–	90.12	90.12	11.71 (13.69)
385–395	–	–	–	47.6	204.4	9.11 (4.61)	–	–	–
375–385	–	–	–	50.8	255.2	18.34 (8.57)	–	–	–
360–375	139.78	297.80	76.32 (33.49)	78.6	333.8	22.88 (16.10)	–	–	–
335–360	–	–	–	91.7	425.5	76.77 (30.46)	42.41	132.53	16.04 (21.04)
320–335	79.85	377.65	96.67 (5.77)	44.8	470.3	90.44 (11.14)	22.37	154.90	21.36 (21.83)
305–320	18.58	396.23	95.24 (8.25)	18.6	488.9	95.86 (4.92)	9.29	164.19	54.67 (34.70)
295–305	–	–		1.3	490.2	88.35 (5.48)	0.74	164.93	65.35 (47.85)

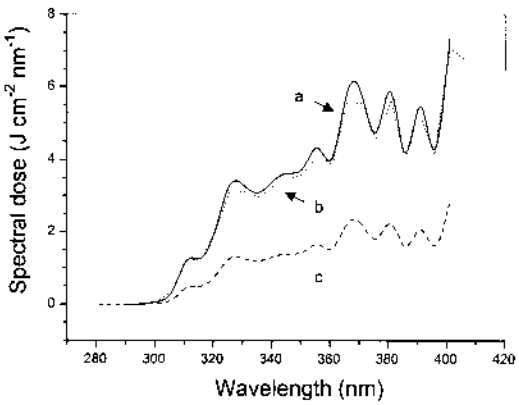

Figure 1. Spectral dose for the three independent UV-exposure experiments ([a] 8–9 January, [b] 17–18 January, and [c] 4–5 February) during the austral summer of 1998.

iments were done under sunny conditions (Fig. 1a,b), while the last one was done under partially cloudy conditions during the last hours of the experiment (Fig. 1c).

Copepod mortality was plotted against dose (biological weighted) in order to assess the dose–response relationship between radiant exposure and mortality in *B. gracilipes*. The survivorship of *B. gracilipes* decreases with increasing weighted dose (Fig. 2).

BWF

BWF for UV-induced mortality in *B. gracilipes* shows decreasing response with increasing wavelengths (Fig. 3). Each BWF value a_i was divided by the corresponding bandwidth, thus making the results bandwidth-independent. In this way, it is possible to compare our results with action spectra (AS) obtained under monochromatic radiation conditions. Setlow's (30) DNA damage and McKinlay and Diffeys's (31) AS are included (Fig. 3). To compare the relative dependence in wavelength, all spectra are normalized to 1.0 at 300 nm.

Discussion

BWF derived for *B. gracilipes* (Fig. 3) shows the typical inverse relationship with wavelength, with the shorter UV-B wavelength being more damaging than UV-A radiation and PAR. Although we do not exactly know the final mechanism responsible for mortality in *B. gracilipes*, a close examination of the slope of the curve shows that BWF for this species more closely resembles the AS for UV-induced erythema in human skin (31) than the Setlow's (30) AS for naked DNA. To our knowledge, the study of Kouwenberg et al. (25) is the only one that allows comparison of BWFs with another zooplankton species. BWF obtained for UV-induced mortality in the eggs of the marine copepod *Calanus finmarchicus* exhibited a slope similar to Setlow's DNA action spectrum (25). The authors suggested that UV-B-induced mortality in *C. finmarchicus* resulted from DNA damage, as no significant effect of UV-A radiation was found. Conversely, in *B. gracilipes* effects of wavelengths less than 360 nm were observed (Table 1). This fact may explain the different slope of BWFs determined for the two copepod species.

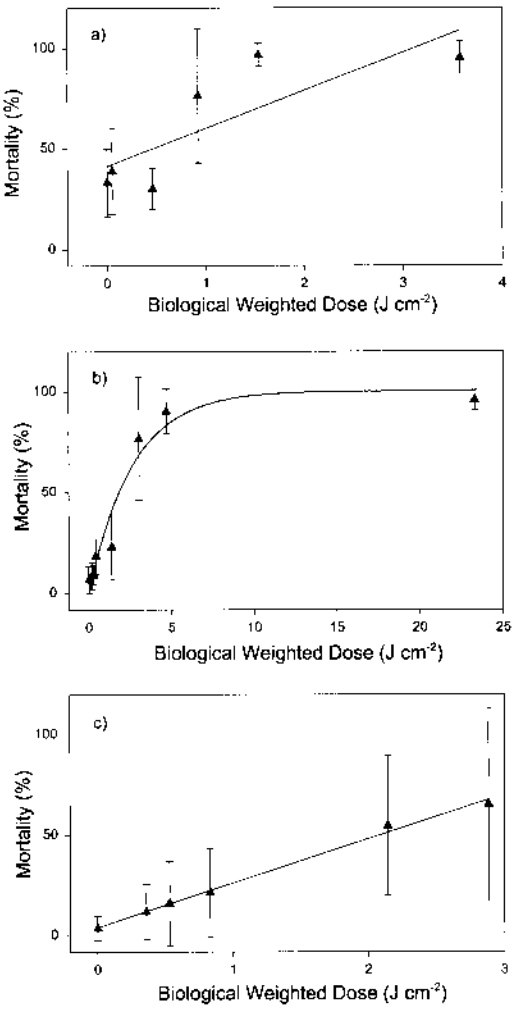

Figure 2. Relative mortality of the calanoid copepod *B. gracilipes* as a function of the biological weighted dose calculated for the (a) 8–9 January, (b) 17–18 January, and (c) 4–5 February UV-exposure experiments. Vertical error bars represent ± 1 standard deviation.

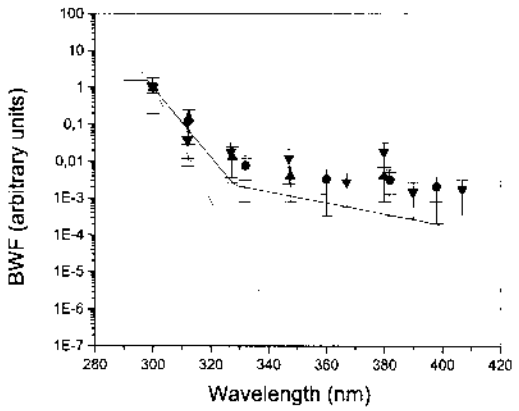

Figure 3. BWF for the mortality of the calanoid copepod *B. gracilipes* (copepodid C V and adults) under solar radiation conditions (▲ 8–9 January; ● 17–18 January and ▼ 4–5 February experiments). AS for wavelength-dependent damage to naked DNA (data redrawn from Setlow [30] [dotted line], and for erythema in human skin [31] [continuous line]). AS were normalized to 1 at 300 nm. Vertical error bars represent ± 1 standard deviation.

For many physiological processes, the chromophores (e.g. polypeptide or ligand molecules that are bound to a large complex) affected by UV radiation are uncertain or unknown (32). Moreover, it is difficult to know how much radiation reaches an intracellular chromophore, as most cellular structures and molecules absorb and scatter radiation (33). Among potential alternative mechanisms of UV damage are direct protein and membrane damage. For example, Ringelberg et al. (19) observed cuticular damage in the freshwater copepod *Acanthodiaptomus denticornis* after UV-exposure. Moreover, indirect oxidative stress can be caused by intra- or extra-organismal formation of ROS. For instance, Warmer and Wei (34) showed oxidative damage in RNA caused by UV-A radiation.

The *B. gracilipes* population considered in this study was sensitive to both UV-B and UV-A radiation ($\lambda < 360$ nm). UV-A sensitivity has often been reported in bacteria and protozoa (35), but is less well documented in metazoa. However, Williamson et al. (14) reported UV-A induced mortality in the cladocerans *Daphnia* and *Diaphanosoma*, and Zagarese et al. (16) had already reported UV-A sensitivity in *B. gracilipes*. Other zooplankton species, such as the calanoid copepod *Diaptomus* or the cladoceran *Chydorus sphaericus*, have been shown to be sensitive to the shorter wavelengths of UV-B, but not to UV-A radiation (14,15).

Dose-dependent effects of UV radiation under laboratory conditions have been reported for several marine copepod species (36–40). The highest spectral resolution of UV-induced damage in copepods is given by Kouwenberg et al. (25). In their study, long-pass filters (Schott) were used in the experiments to determine the mortality in *C. finmarchicus* eggs under sunlamps (Xenon-arc-lamp). The strongest effects were found under exposure of this species to wavelengths below 312 nm. At the shortest wavelengths (< 305 nm), UV-induced mortality was strongly dose-dependent. However, survival was higher when exposed to 312 nm, and exposure to wavelengths of 335, 360 and 400 nm showed no effect. Dose-dependent mortality was also shown in *B. gracilipes*. The strongest effects occurred under exposure to wavelengths below 335 nm, survival increased when wavelengths shorter than 360 nm were filtered out. Exposure to wavelengths longer than ~360 nm showed no effects.

BWFs are likely to differ among different organisms. Among the factors that may affect the shape of the BWF are presence of photoprotective pigments or activity of repair mechanisms. Previous studies by Zagarese and co-workers (16) have shown that the *B. gracilipes* populations of northwestern Patagonian lakes have a rather limited capacity of photorepair (the mechanism that monomerizes cyclobutane pyrimidine dimers catalyzed by the enzyme DNA photolyase in the presence of UV-A and blue-light) (41). Exposure to a wide range of natural radiation has shown that UV-induced mortality in *B. gracilipes* obeys the principle of reciprocity (29,42), *i.e.* the amount of damage is a function of dose for a wide range of irradiance and time scales. We believe that the adherence to reciprocity is due to the species' lack of an efficient repair mechanism. Our choice of the species was based on this characteristic (reciprocity) that allows that data from different experiments could be combined.

A potential shortcoming of using natural radiation to de-

rive a BWF is that the use of cut-off filters permits the progressive removal of the shorter wavelengths, but not the opposite. This fact generates an asymmetry in the data matrix, since the values above the diagonal are all zero. Therefore, it is conceivable that our estimations of BWF could be biased. Although this is not the best approach, it resembles field conditions where the downwelling flux of radiation is progressively depleted of the shorter wavelengths with depth.

The advantage of using whole organisms and natural radiation is that the exposure conditions more closely resemble the typical exposure experienced in nature, and are therefore more realistic than using monochromatic radiation and/or parts of an organism (naked DNA, membranes, etc.). The trade-off of doing experiments with natural UV radiation is that effects can no longer be attributed to specific wavelengths. The weights are therefore the sum of effects at a given wavelength and the interactive effects of other wavelengths (43).

Previous experiments showed that the mortality of vertically moving *B. gracilipes* individuals could be predicted by the mortality observed in individuals exposed at fixed depths (29,42). Therefore, we believe that the BWF derived in this study applies to natural conditions as long as the dose experienced by the organisms could be calculated. Although *B. gracilipes* occurs in a variety of lakes differing in water transparency and mean depth, BWF derived here may be useful to estimate the UV risk in different environments. This may particularly be useful for well-mixed shallow lakes, where avoidance of UV in deep water may be prevented by turbulent mixing induced by strong winds. In deeper lakes, BWF may still be useful for defining the depth where the population may be affected by UV radiation.

Acknowledgments—We thank Don Morris and Bruce Hargreaves for providing the Schott filters and the PUV radiometer, Victor Baez for permitting us to do the experiments at the fish-farm, Jorge Chiapella, Mónica Díaz and Chris Osburn for their assistance in the field, and Ruben Sommaruga for critical comments to the manuscript. This research was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (Convenio de Cooperación Internacional CONICET-NSF, Expediente N° 1349/98) and by the International Foundation for Science (grant A/2325-2). B.T. was partially supported by the Austrian Research Association.

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