

UV-B-induced damage and photoreactivation in three species of *Boeckella* (Copepoda, Calanoida)

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Abstract. Solar ultraviolet (UV) radiation poses a threat to most living organisms. Aquatic organisms have evolved three basic mechanisms to cope with harmful levels of radiation. Two mechanisms, avoidance (e.g. vertical migration) and photoprotection (e.g. production of photoprotective compounds that act as filters, antioxidants, etc.), serve to minimize the dose of UV radiation that reaches the organism's vital structures (DNA, membranes, etc.). The third mechanism, repair (e.g. dark repair mechanisms, such as nucleotide excision repair; or photoreactivation mechanisms, such as photoenzymatic repair), serves to repair the damage following UV exposure. Here, we compare the vulnerability to UV-B radiation of three copepod species (*Boeckella brevicaudata*, *Boeckella gibbosa*, and *Boeckella gracilipes*) that occur in lakes that differ in UV-B penetration and depth. Our aim was to gain insight into the significance of each of the three mechanisms in different UV-B environments. Results from a 3-day 'in situ' incubation in ultra-oligotrophic Lake Toncek showed that *B. gracilipes* is highly vulnerable to UV-B and UV-A radiation. In contrast, virtually no mortality was observed in *B. gibbosa* and *B. brevicaudata* during the same period. In order to discriminate the contribution of photoprotection and photoreactivation, the three species were subsequently exposed in the laboratory to an artificial source of UV-B radiation, both in the presence and absence of visible radiation (recovery radiation). The photoprotection potential (i.e. resistance to UV-B in the absence of recovery radiation) of *B. gracilipes* and *B. gibbosa* was lower than that of *B. brevicaudata*. On the other hand, photoreactivation (higher resistance to UV-B in the presence of recovery radiation) was observed in *B. brevicaudata* and *B. gibbosa*, but not in *B. gracilipes*. To cope with damaging UV-B levels in nature, *B. gracilipes* depends exclusively on the attenuation by the external media (i.e. avoidance). Although *B. gibbosa* tends to avoid the surface waters of lakes, it also occurs in shallow transparent pools. Most likely its ability to survive in these shallow, high UV environments is due to its photoreactivation potential. Finally, despite its occurrence in highly turbid lakes, *B. brevicaudata* seems extremely well suited to cope with UV-B radiation thanks to a combination of photoreactivation and photoprotection.

Introduction

Solar ultraviolet (UV) radiation has long been suspected to influence the distribution of zooplankton in freshwaters. Earlier investigators (Brehm, 1938; Thomasson, 1956) speculated on the role of pigmentation and suggested that high levels of solar UV radiation in alpine environments were responsible for the scarcity of planktonic species in highly exposed habitats. Recent research on UV photobiology has been stimulated by the prospects of future UV-B increases due to anthropogenic depletion of stratospheric ozone. Most efforts in recent years have been oriented towards marine ecosystems and phytoplankton communities. Nevertheless, the accumulated evidence supports the major assumptions by earlier freshwater zooplanktologists: UV-B penetrates to biologically significant depths in oligotrophic (oceanic or fresh) waters (Morris *et al.*, 1995); many zooplankton species are vulnerable to natural levels of UV radiation (Williamson *et al.*, 1994); and the organisms from highly exposed habitats are generally more resistant to UV-B than those occurring in less exposed environments.

Aquatic organisms can reduce the negative effects of solar radiation by means of three basic mechanisms: avoidance (e.g. vertical migration), photoprotection (e.g. presence of compounds that serve as filters or antioxidants), and repair (e.g. enzymatic DNA repair, etc.) (Siebeck *et al.*, 1994; Zagarese and Williamson, 1994). Vertical migration is known to be induced by the presence of predators (Dodson, 1988; Dawidowicz and Loose, 1992; Dawidowicz, 1993), but regardless of its ultimate cause, this migratory behavior effectively protects the zooplankton from damaging levels of UV-B radiation (Zagarese *et al.*, 1994). Vertical migration is restricted to lakes that are deep enough relative to their transparency. The presence of photoprotective compounds is generally associated with higher tolerance to solar radiation, and seems to be restricted to environments without visual predators (Luecke and O'Brien, 1981). Repair mechanisms (particularly enzymatic DNA repair) are widespread across many types of organisms (Karentz *et al.*, 1994). The ecological significance, costs, and constraints of repair are poorly understood. The latter is particularly true for those mechanisms that are activated by longer wavelength radiation (photoreactivation), because the ratio between UV-B and recovery radiation (380–450 nm) varies dramatically with depth, as well as across environments. In freshwater zooplankton, photoreactivation (presumably photoenzymatic DNA repair) has been demonstrated in *Daphnia* (Siebeck, 1978; Siebeck and Böhm, 1991), but attempts to demonstrate its presence in copepods have failed (Ringelberg *et al.*, 1984). The paucity of available information on photoreactivation in freshwater zooplankton does not allow generalizations on patterns across environments.

The objective of this study is to compare three species of the genus *Boeckella* (Copepoda: Calanoida) with regard to their tolerance to UV-B radiation, both in the presence and absence of recovery radiation (i.e. longer wavelength radiation). The genus *Boeckella* is widely distributed and often dominates the zooplankton communities of lakes in Patagonia, across a wide range of physical (particularly optical), chemical and biological variables. Despite the wide distribution of the genus, the three species considered in this study seldom co-occur, suggesting specific adaptations to their different environments.

Method

Lakes and zooplankton

The translucent *Boeckella gracilipes* Daday is typical of deep ultra-oligotrophic lakes (where it usually remains deep during the day), but also occurs in shallower, more productive water bodies (personal observation). The red colored *Boeckella gibbosa* (Brehm) is typical of ponds and lakes above the timberline (Modenutti, 1993, and unpublished data), which are usually ultra-oligotrophic and relatively shallow (from a few centimeters to ~20 m). Finally, *Boeckella brevicaudata* (Brady) occurs in temporary, shallow lakes (0.5 to a few meters) characterized by high UV and PAR attenuation. *Boeckella brevicaudata* is dark pigmented with colors varying from burgundy to blue.

Specimens for experiments were collected from lakes Toncek (*B.gibbosa*), Los Juncos (*B.brevicaudata*), and Ezquerria (*B.gracilipes*). Lake Toncek (41°12'S,

71°29' W) is a clear ($Z_{1\%,305} = 1\%$ attenuation depth for $\lambda = 305$ nm = 6.4 m; estimated with the PUV-500) ultra-oligotrophic lake located in Cerro Catedral at 1700 m above sea level. Maximum depth is 12 m, and the zooplankton community is dominated by *B.gibbosa*. Los Juncos (41°4'S, 71°0'W) is a shallow (Z_{\max} during the study period = 0.6 m), semi-permanent, highly productive pool ($Z_{1\%,305} = 0.09$ m; estimated with the PUV-500). Lake Ezquerra (41°3'S, 71°31'W) is intermediate in transparency ($Z_{1\%,305} = 1.27$ m; estimated from spectrophotometric absorbance) and depth ($Z_{\max} = \sim 3$ m).

'In situ' incubation

We incubated the three species of *Boeckella* at 0.5 m depth in Lake Toncek. Twenty adult individuals were placed in quartz tubes (volume: 40 ml, inner diameter: 15 mm) filled with water from the 'home' lake and additions of *Chlamydomonas reinhardtii* from a culture (final density ~ 750 cells ml⁻¹). The tubes were closed with silicon stoppers. One third of the tubes was covered with Mylar® D (Dupont Corp.; thickness = 0.023 mm) to filter out the UV-B range of the solar spectrum, another set was left uncovered, and the last set was wrapped with aluminum foil (dark controls). Each combination of species and radiation treatment consisted of five replicates, totaling 45 tubes (3 species \times 3 treatments \times 5 replicates). The copepods were collected from the field within 24 h from the beginning of the incubation. The tubes containing *B.gracilipes* or *B.brevicaudata* were set up in the lab and transported to Lake Toncek in dark, thermally insulated containers. The tubes containing *B.gibbosa* were set up at Lake Toncek immediately before the incubation. The tubes were placed in the lake on 20 January 1995 at 20:00 h and retrieved after 70 h. The copepods were counted in the field under a dissecting scope immediately after the end of the experiment, and subsequently preserved in formalin (4% final concentration). The number of dead and live animals was recorded. Surface and underwater UV and PAR measurements were made with a PUV-500 submersible profiling radiometer (Biospherical, Inc.) and an IL 1700 broad band submersible radiometer (International Light, Inc.).

Exposure to an artificial source of UV-B

General experimental conditions. All experiments were run in an incubator at 15°C. Each experimental unit consisted of 20 adult copepods placed in a 55-mm Pyrex Petri dish filled with 15 ml of filtered (0.2 μ m) water from the 'home' lake. The Petri dishes were placed on a built-in, clear acrylic turntable rotating at 1 r.p.m., and arranged equidistantly from the center. A Spectroline XX15-B fluorescent lamp (Spectronics Corp.) provided the source of UV-B radiation. The lamp was covered with a sheet of cellulose diacetate to remove wavelengths shorter than ~ 295 nm (Figure 1). The acetate sheet was replaced before each experiment to minimize between-experiment differences due to optical degradation. The duration of the UV-B exposure was 4 h in all experiments. Different UV-B intensities were obtained either by changing the distance from the lamp (from 23 to 47 cm, in different experiments) or by covering the individual Petri dishes with a

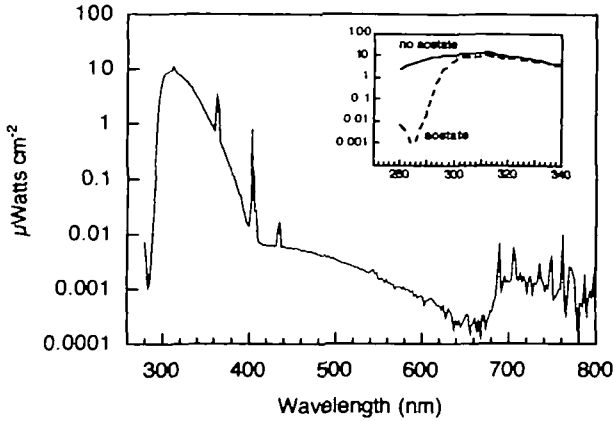


Fig. 1. Spectral composition of the radiation emitted by the Spectronic XX15B lamp measured with an OL 752-PMT scanning spectroradiometer (Optronics Laboratories) from a distance of 23 cm. The sheet of cellulose acetate served to remove wavelengths shorter than about 295 nm (small panel). Visible radiation measured with the IL-1700 radiometer was $\sim 3 \times 10^{-6} \mu\text{E cm}^{-2} \text{ s}^{-1}$. For the photorepair experiments, the visible light radiation was 3 orders of magnitude higher ($2.7 \times 10^{-3} \mu\text{E cm}^{-2} \text{ s}^{-1}$).

variable number of layers of plastic window screen (for different treatments within the same experiment). Visible radiation was provided by two 120 cm (Philips daylight, TLT 40 W/54RS), and two circular 30 cm diameter (Toshiba daylight, FCL 32D/30 W) fluorescent tubes. Visible and UV radiation were measured with an International Light, Inc. IL 1700 radiometer equipped with interchangeable UV-B, UV-A, and PAR detectors. These measurements provided UV-B estimates that allowed us to compare the results from different experiments using the same lamp. They were not intended, however, for comparisons to different radiation configurations or to natural conditions.

Exposure to artificial UV-B radiation 'in the dark'. The objective of these experiments was to compare the photoprotection of the three *Boeckella* species (i.e. the resistance to UV-B in the absence of recovery radiation). Each experiment had five UV-B treatments (i.e. UV-B intensities or dose-rates), and a dark control (fully wrapped with aluminum foil). Each treatment had three replicates. After exposure to UV-B radiation for 4 h the animals remained in the dark for 20 additional hours. In no case was the incubator opened before 24 h from the beginning of the incubation. After 24 h the animals were inspected under a dissecting microscope and the number of dead and live individuals recorded. The copepods were subsequently moved inside a second incubator (also set at 15°C) with a 14:10 light:dark cycle for an additional 24 h period, and recounted. Only the results based on counts after 48 h are reported, because the counts after 24 h were ambiguous due to the presence of 'moribund' individuals.

Exposure to UV-B in the presence of recovery radiation. The objective of these experiments was to compare the photoreactivation of the three species of

Boeckella (i.e. the survival of animals exposed to UV-B and recovery radiation). The experimental set-up was similar to the experiments 'in the dark', except that the copepods received UV-B and visible radiation simultaneously for 4 h. After UV-B exposure, the animals continued receiving visible radiation for at least 10 more hours (the effectiveness of visible radiation decreases sharply after ~2 h from UV-B exposure, Siebeck and Böhm, 1994). The following 24-h period was identical to the 'dark' experiments. Because we could not vary the exposure to visible radiation while keeping constant the intensity of UV-B, only two treatments, (1) UV-B + visible radiation and (2) dark controls, were run in each trial.

'Double-check' experiments. In order to compare results obtained on different days, we must assume that differences between trials are due only (or at least primarily) to treatment differences, but not to differences in the physical environment (i.e. lamp output, temperature) or in the physiological state of the animals. Variations in the physical environment were minimized by always using the same incubator and lamp (which had been burnt for 100 h before the first trial) and new acetate sheets for each trial. Routine measurements of the lamp output indicated that it did not vary appreciably, but we must admit that a broad-band radiometer, such as the IL 1700, would not detect variations in spectral composition. Similarly, several precautions were taken to minimize the risk that differences between individual copepods could bias the results: (i) all trials included dark control treatments, (ii) treatments in different trials were run in an alternated sequence (i.e. alternating low and high doses), (iii) all experiments were run using only seemingly healthy animals collected within 2–3 days. In addition, we ran abbreviated versions of previous experiments to verify the consistency of the results obtained on different days. Most of these 'double-check' experiments are not reported here, because they only served to check the methodology, but provided no additional information.

Statistical analysis

The results from the 'in situ' incubation were analyzed with individual ANOVAs for each of the three species, and 'a posteriori' test of means comparisons (Scheffé). The proportion of dead individuals was transformed to the arcsin of the square root (Sokal and Rohlf, 1981). LD₅₀ values were obtained in the lab experiments by fitting the experimental data to a Probit model (Finney, 1971).

Results

'In situ' incubation

The three species showed a low proportion (<5%) of dead individuals in the control (foil wrapped) treatments. *Boeckella brevicaudata* and *B.gibbosa* showed negligible mortality, both in the Mylar and the quartz treatments. In contrast, the mortality experienced by *B.gracilipes* was 32.7% in the Mylar treatment, and as much as 79.1% in the quartz treatment (Figure 2, Table I). Ground level UV-B irradiance at noon averaged 63 $\mu\text{W cm}^{-2}$ as measured with the IL 1700 broad band

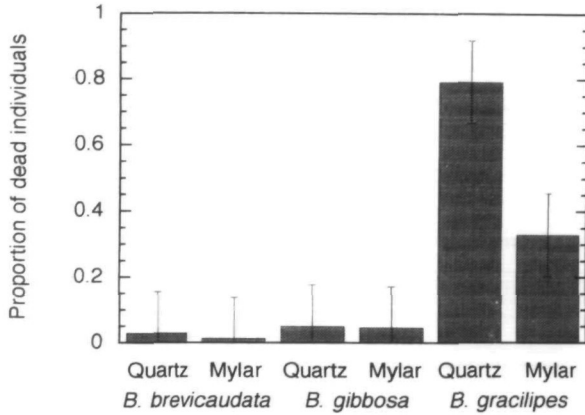


Fig. 2. Relative mortality (mortality in the treatments minus mortality in the dark controls) of three species of *Boeckella* after a 3-day incubation at 0.5 m in Lake Toncek. The mortality in the control treatment (not shown) was under 5% for the three species. Vertical bars: 1 standard error.

radiometer (spectral range 275–310 nm according to manufacturer’s specifications). Spectral irradiance measured with the PUV-500 averaged $3.8 \mu\text{Watts cm}^{-2} \text{nm}^{-1}$ (for the band centered at 305 nm). (For a comparison of the two instruments see Kirk *et al.*, 1994.)

Exposure to artificial UV-B radiation

Exposure to UV-B ‘in the dark’. The shape of the relationship between the proportion of dead individuals and the intensity (dose-rate) of UV-B radiation was sigmoid for the three species of *Boeckella*. However, *B.brevicaudata* ($LD_{50\text{dark}}: 124.5 \mu\text{W cm}^{-2}$ measured with the IL-1700 radiometer) proved to be over an order of magnitude more resistant than either *B.gracilipes* ($LD_{50\text{dark}}:$

Table I. Separate analyses of variance on the proportion of dead individuals after incubation in Lake Toncek for the three species of *Boeckella*. The proportion of dead individuals was transformed as the arcsin of the square root before the analyses

Species	Number of tubes	F	P
<i>B.brevicaudata</i>	15	0.5197	0.6075
<i>B.gibbosa</i>	15	1.2804	0.3133
<i>B.gracilipes</i>	15	82.0192	<0.0001

Scheffé test for *Boeckella gracilipes*

Matrix of pairwise comparison probabilities

	Control	Quartz	Mylar
Control	1.0000		
Quartz	<0.0001	1.0000	
Mylar	<0.0001	0.0048	1.0000

8.8 $\mu\text{W cm}^{-2}$) or *B.gibbosa* ($\text{LD}_{50\text{dark}}$: 7.6 $\mu\text{W cm}^{-2}$). The latter two did not differ statistically (Figure 3, and Table II).

Exposure to UV-B in the presence of recovery radiation. For *B.gibbosa* and *B.brevicaudata*, the proportion of dead individuals exposed to UV-B and visible radiation was significantly lower than when exposed to the same intensity of UV-B in the absence of visible radiation. The LD_{50} value of *B.gibbosa* in the presence of visible radiation ($\text{LD}_{50\text{light}}$: 58.1 $\mu\text{W cm}^{-2}$) was significantly higher than 'in the dark' (Table II). Because of the high tolerance to UV-B radiation exhibited by *B.brevicaudata* in the presence of visible radiation, and our inability to produce higher UV-B intensities, we were unable to estimate the $\text{LD}_{50\text{light}}$ for *B.brevicaudata*. Nevertheless, the available data indicate the $\text{LD}_{50\text{light}}$ for *B.brevicaudata* must be higher than 271 $\mu\text{W cm}^{-2}$, since negligible mortality was observed when the organisms were exposed to visible radiation during UV-B exposure at a dose rate of 271 $\mu\text{W cm}^{-2}$ (Figure 3).

Unlike the other two *Boeckella* species, the mortality of *B.gracilipes* was similar in the presence and in the absence of visible radiation (Figure 3), suggesting little, if any, photoreactivation. Although the available data do not allow us to make an independent estimation of the $\text{LD}_{50\text{light}}$, the similarity in the response of *B.gracilipes* with and without visible radiation suggests that the $\text{LD}_{50\text{light}}$ and $\text{LD}_{50\text{dark}}$ are also rather similar.

Discussion

The field experiment confirmed that current summer levels of solar radiation at 41°S are lethal to sensitive zooplankton species, such as *B.gracilipes*. In addition, the differences between quartz and Mylar treatments showed that both, UV-A and UV-B, contribute to the observed mortality in this species. On the other hand, under rather similar exposure conditions *B.brevicaudata* and *B.gibbosa* experienced virtually no mortality. In principle, these results are consistent with the generally accepted hypothesis that pigmented species are less vulnerable to UV radiation than unpigmented species (Hairston, 1979; Byron, 1982; Ringelberg *et al.*, 1984; Hebert and Emery, 1990; Siebeck and Böhm, 1994). However, when exposed to an artificial source of UV-B radiation in the absence of recovery radiation (i.e. 'in the dark'), *B.gibbosa* was as sensitive as *B.gracilipes*. In other words, the presence of pigments (most likely carotenoids) responsible for the red coloration of *B.gibbosa* does not seem to improve its tolerance to UV-B radiation in the absence of longer wavelength light. These results are consistent with the idea that carotenoid pigments do not act as UV-B filters (Hessen, 1994). On the other hand, the higher tolerance to UV-B shown by *B.brevicaudata*, both in the presence and in the absence of visible radiation, may be a consequence of its larger size or the presence of a different type of pigment.

The recovery effect of visible radiation (photoreactivation) is considered a universal phenomenon in animals (Siebeck and Böhm, 1991, 1994; Siebeck *et al.*, 1994), algae, bacteria, and other organisms (Karentz *et al.*, 1994). In our experiments, *B.gibbosa* and *B.brevicaudata* showed increased survival when exposed

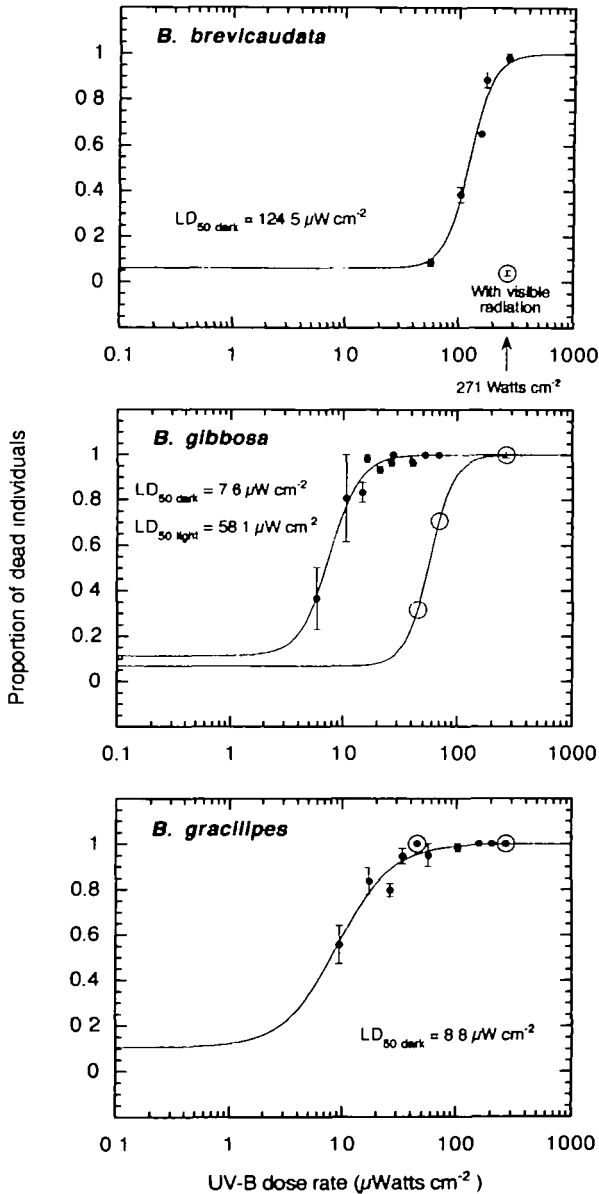


Fig. 3. Mortality versus UV-B intensity curves for the three species of *Boeckella*. Exposure duration: 4 h. Dark symbols: exposure to UV-B 'in the dark'; open symbols: simultaneous exposure to UV-B and visible light. Vertical bars: 1 standard error. Control treatments (i.e. UV-B intensity = 0) were excluded from the figure to allow plotting on a log scale, but were considered for estimating LD₅₀.

to an artificial source of UV-B radiation in the presence of visible radiation. The dose modification factor (under our experimental conditions) estimated for *B.gibbosa* (DMF = 7.6) is in the range of values reported for *Daphnia* (Siebeck

Table II. LD₅₀ values for the three species of *Boeckella* exposed to artificial UV-B radiation for 4 h in the presence and absence of visible radiation

	Without visible radiation LD _{50dark} ($\mu\text{W cm}^{-2}$)		With visible radiation LD _{50light} ($\mu\text{W cm}^{-2}$)	
	Mean	95% confidence interval	Mean	95% confidence interval
<i>B. brevicaudata</i>	124.5	110–136	>271	N/A
<i>B. gibbosa</i>	7.6	4.2–10.1	58.1	51.8–64.4
<i>B. gracilipes</i>	8.8	5.8–11.5	(*)	N/A

(*) Presumably LD_{50light} \approx LD_{50dark} for *B. gracilipes* (see text and Figure 3).

and Böhm, 1991). On the other hand, the DMF for *B. brevicaudata* is surely higher than 2.1 (a rather conservative estimate considering our failure to estimate the LD_{50light} for this species). In contrast, under identical experimental conditions, *B. gracilipes* showed no signs of photoreactivation. Moreover, the differences in mortality between *B. gracilipes* and *B. gibbosa* when exposed to UV-B and recovery radiation can be accounted for by the lack of photoreactivation in *B. gracilipes*. Similar results have been reported for *Daphnia* (Siebeck and Böhm, 1991). *Daphnia galeata* and *Daphnia pulex obtusa* showed similar tolerance to UV-B exposure in the absence of recovery radiation, but the photoreactivation potential was several times higher in the second species (DMF 4.99 versus 14.70).

Previous studies (Ringelberg *et al.*, 1984) failed to demonstrate photoreactivation in copepods, but these authors attributed the 'apparent' lack of photoreactivation to experimental errors. Ringelberg and coworkers compared the survival of *Acanthodiaptomus denticornis* individuals that were either kept in the dark or placed in the light after UV exposure. The authors recognized that the fact that all individuals (regardless of treatment) were exposed to a minimal dose of visible radiation during handling (~ 15 min) was a potential source of error in their experiments. Ringelberg *et al.* reasoned that 'since many animals show photoreactivation . . . and since it is considered a universal phenomenon also in plants . . . , an experimental error on our part is the most plausible explanation [for the lack of differences in survival between copepods irradiated with and without visible radiation]'. They subsequently raised the possibility that even a short exposure to dim light could suffice to activate fully the photoreactivation mechanism: 'Since the animals had been exposed to the light for at least a quarter of an hour twice daily in order to check on mortality, it may be possible that all our animals were photoreactivated'. We have not attempted to test this hypothesis formally, but in preliminary, replicated trials with *B. gibbosa* we found that exposure to a UV-B intensity of $28 \mu\text{W cm}^{-2}$ for 4 h caused total mortality if the copepods received no visible radiation, but virtually no mortality if they were exposed to visible radiation ($2.8 \times 10^{-4} \mu\text{E cm}^{-2} \text{ s}^{-1}$) for ~ 15 min immediately after UV-B exposure. Neither these results nor Ringelberg *et al.*'s previous study can be considered definitive, but they warn us against unwanted exposure to longer wavelength radiation in photoreactivation experiments.

The responses of aquatic animals to UV radiation are highly dependent on the spectral composition of the source, its intensity (or dose-rate), and the exposure duration. Because of differences in spectral composition and exposure duration, a direct comparison of the results from field and lab experiments is not possible. To circumvent this limitation we may compare field and lab results using the mortality of *B.gracilipes* as a common currency. This procedure should be equivalent to using the (unknown) biological weighting function of *B.gracilipes* to weigh raw radiation measurements. We know for instance, that the UV dose in the field incubation caused 79.1% of mortality in *B.gracilipes*. On the other hand, the dose required to kill 79.1% of *B.gracilipes* in the lab produced negligible mortality in the other two species when recovery radiation was administered simultaneously. From these results we could have predicted the almost negligible mortality of *B.gibbosa* and *B.brevicaudata* observed in the field (Figures 2 and 3).

To cope with potentially damaging UV-B radiation, *B.gracilipes* seems to depend exclusively on the attenuation by the external media: occurring below 9–18 m in transparent lakes, or in relatively turbid shallow lakes (unpublished data). *Boeckella gibbosa* also tends to avoid the topmost meters in Lake Toncek (Guerrero, 1993), but avoidance is unfeasible in shallow, transparent pools. We were surprised by the relatively low UV-B photoprotection observed in *B.gibbosa*. The survival of this species in the absence of a depth refuge (shallow pools) appears to depend almost exclusively on photoreactivation. Finally, despite its occurrence in highly turbid lakes, *B.brevicaudata* seems extremely well suited to cope with UV-B radiation thanks to a combination of photoprotection and photoreactivation. We believe that strong turbulence in shallow lakes (MacIntyre, 1993) or the seasonal drying of temporary pools may force the organisms to the highly exposed topmost centimeters of water.

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