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Ultraviolet damage and counteracting mechanisms in the freshwater copepod *Boeckella poppei* from the Antarctic Peninsula

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Abstract

The process of ozone depletion over the Antarctic continent has resulted in the increase of incident ultraviolet-B (UVB) radiation, whose effects may be damaging to living organisms. To counteract the negative effects of ultraviolet radiation (UVR), aquatic organisms may display one or more strategies: (1) avoidance (i.e. deep distribution); (2) photoprotection through the use of ''sunscreen'' compounds, such as mycosporine-like amino acids (MAAs); and (3) enzymatic repair of the damage. The effects of UVR were assessed on four populations of the copepod *Boeckella poppei* from Antarctic lakes using laboratory and field experiments. The results were related to measurements of DNA enzymatic repair activity and MAA concentration. This is the first study that combines these measurements in zooplankton. *Boeckella poppei* was highly tolerant to UVR (LD_{50} = 2.2–2.78 J cm^{-2}). However, measurements of photorecovery (comparison of UVB mortality in the presence and absence of photoreactivating light) and dosage of photolyase activity indicated low rates of enzymatic repair, which may be the result of low temperatures typical of Antarctic lakes. Three different MAAs were identified, both in phytoplankton and copepods: porphyra-334, mycosporine-glycine, and shinorine. The population of *B. poppei* from Lake Boeckella had the lowest MAA concentration, as well as the lowest tolerance to artificial and natural UVR. These findings support the idea that UV tolerance in this species is related to the accumulation of MAAs. A comparison of the strategies used to cope with potentially damaging levels of UVR by different species of *Boeckella* indicates a high degree of plasticity in this genus, which has probably been key for its success to colonize a wide range of UV environments.

The thinning of the ozone layer over the Antarctic continent (Farman et al. 1985) resulted in substantial increases in ground level fluxes of ultraviolet radiation, particularly within the ultraviolet-B (UVB) region (290–320 nm) of the solar spectrum. Many studies have documented the inhibitory effect of enhanced ultraviolet radiation (UVR) on marine organisms from Antarctic waters (Vernet and Kozlowski 2001). However, fewer studies have assessed the effect of UVR on freshwater organisms from Antarctic lakes (Cabrera and Pizarro 1994; Kepner et al. 2000).

Aquatic organisms have evolved three basic strategies to counteract the negative effects of solar UVR (Zagarese and

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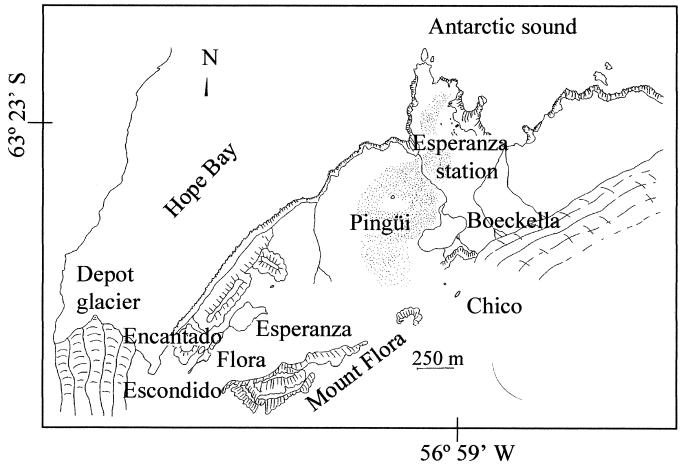

Fig. 1. Site map showing the location of the studied lakes.

Williamson 1994). In many instances, the organisms may avoid being exposed to damaging fluxes of UVR by remaining deep in the water column during the day. Such an avoidance strategy may not be feasible in cases where the waterbody is relatively shallow and the water is highly transparent to UVR. Strong vertical mixing of the water column driven by wind-induced turbulence is an additional factor that may lower the effectiveness of the avoidance strategy (Zagarese et al. 1998). Preliminary search of the available literature on lakes located in the Antarctic Peninsula (Izaguirre et al. 1998) suggested that they are in fact highly exposed to UVR due to the combined effects of shallowness, transparency, and exposure to extremely strong winds.

A second mechanism to cope with high UVR exposure is the synthesis or acquisition of UV-protective compounds that act either as UV sunscreens or as antioxidants. Among these compounds, the so-called mycosporine-like amino acids (MAAs) are widespread among benthic and planktonic marine organisms (Roy 2000). MAAs are water-soluble compounds having high absorption efficiency in the UV range (absorption maxima 309–360 nm). Recently, MAAs have also been found in freshwater copepods and rotifers, but not in cladocerans (Sommaruga and Garcia Pichel 1999; Tartarotti et al. 2001).

The last line of defense against UVR exposure consists in repairing the damage after it has occurred. By far, the most common type of DNA damage induced by ultraviolet-C (UVC) and UVB is the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (Friedberg et al. 1995). These lesions block DNA replication and transcription leading to mutagenic or lethal effects. Living organisms have evolved a specific mechanism for the repair of pyrimidine dimers, called photoreactivation. This mechanism involves a single enzyme (photolyase), which directly reverses the pyrimidine dimers into the two pyrimidine nucleotides by using the energy of near ultraviolet and visible light (Todo et al. 1993; Friedberg et al. 1995). Photolyase activity is widely distributed in nature, and photoreactivation has been found in members of all kingdoms of life. Nevertheless, this mechanism is absent in many species (Sancar 1994).

This paper reports on the mechanisms used by *Boeckella poppei* to cope with increased levels of UVR in shallow freshwater lakes located in the Antarctic Peninsula. The genus *Boeckella* has a circumpolar distribution (Menu Marque et al. 2000) and is one of the dominant species in lakes from Australasia, Antarctica, and South America.

Materials and methods

The study area includes several freshwater lakes and ponds near Base Esperanza (Argentina) in the Antarctic Peninsula at Hope Bay (63°S, 57°W; Fig. 1). These waterbodies (hereinafter referred to as ''lakes'' for simplicity) are covered by a thick layer of ice and snow during most parts of the year. Light conditions below the ice cover are dim (Kepner et al. 2000), but after ice-break, the organisms are suddenly exposed to higher UVR and long photoperiods. Thus, even if the instantaneous fluxes of UVR are not too high, the total dose may be high because of the duration of the daylight (Merilä et al. 2000). A group of seven lakes were sampled during summer 2000 (Table 1). UV exposure experiments were performed on copepods from four lakes (Boeckella, Chico, Esperanza, and Flora).

Ground-level solar radiation was measured during the study period using an IL-1700 radiometer (International

Table 1. Summary of characteristics of the studied lakes. Values of lake altitude, maximum depth, and temperature are from Izaguirre et al. (1998).

	Boeckella	Chico	Encantado	Escondido	Esperanza	Flora	Pingüi
Altitude (m a.s.l.)	49	100	55	50	52	52	20
Maximum depth (m)	4	5.5	~1	~1	4.8	7	~1
Surface temperature (°C)	0.5–4.7	0–2.5	0–10.9	0–1.1	0.3–11.1	0–6.4	0–17
K_d PAR (m^{-1})	4.4	0.96	7.1	2.22	na	na	na
K_d UVA (m^{-1})	7.24	1.44	0.74	2.99	0.92	0.64	na
a_d at 320 nm (m^{-1})	1.39	0.91	0.72	0.42	0.67	0.42	102.63
a_d at 380 nm (m^{-1})	0.68	0.46	0.27	0.18	0.32	0.18	46.1
DOC (mg L^{-1})	0.68	0.48	0.87	0.48	0.48	0.38	41.9
Chl *a* (μg L^{-1})	0.30	0.78	0.31	0.46	0.27	na	2,403.5
Seston MAA (μg L^{-1})	0.0016	0.027	na	na	0.009	0.0058	na
MAA/Chl *a* (μg μg^{-1} Chl *a*)	0.0053	0.035	na	na	0.034	na	na
Occurrence of *Boeckella poppei*	Yes	Yes	Yes	No	Yes	Yes	No

Light) equipped with four sensors (PAR, UVA, UVB, and actinic). In situ underwater measurements of solar radiation were taken using the IL-1700 radiometer (for a detailed description of the instrument *see* Kirk et al. 1994). The diffuse attenuation coefficient (K_d) was calculated as the slope of the relationship ln(irradiance) versus depth (m^{-1}). Concurrent with field optical data, samples of water were collected for chemical and optical analyses. Chlorophyll *a* (Chl *a*) was determined by the spectrophotometric method of Nusch (1980) and Marker et al. (1980). Briefly, samples were filtered onto Whatman GF/F filters and the pigments were extracted with hot 90% ethanol. Correction for pheopigments was performed by acidification with 0.1 N HCl. The same filtering protocol was used for the collection of seston samples for MAA analysis (*see below*). Dissolved organic carbon (DOC) concentration was measured by the high-temperature Pt catalyst oxidation method (Shimadzu TOC-5000) following the recommendations of Sharp (1993). Spectrophotometric absorption coefficients (a_d) of filtrated samples (Sterivex, 0.22-μm filters) were determined every 1 nm from 250 to 750 nm using 1- or 10-cm quartz (Suprasil) cuvettes and a Hewlet-Packard 8453 spectrophotometer. These measurements were referenced against deionized distilled water. Absorption coefficients were calculated as $a_d = \ln(10^A)$ and reported for a 1-m path (m^{-1}) at 320 and 380 nm.

Zooplankton was collected using a 55-μm-mesh plankton net. *Boeckella poppei* individuals were counted and sorted under a dissecting scope. Samples were preserved in 4% formalin for taxonomic identification. In addition, a prescribed number of individuals were stored at −20°C for MAA analysis and photolyase activity determinations (*see below*).

To determine the presence of UV-absorbing compounds in *B. poppei*, 100 individuals were extracted in 20% aqueous methanol (v/v; MeOH) for 2 h in a water bath at 45°C (García Pichel and Castenholtz 1993; Sommaruga and Garcia Pichel 1999). The extracts were scanned in a diode array, UV-visible (UV-Vis) spectrophotometer (Hewlet-Packard 8453) between 250 and 750 nm using a 1-cm-pathlength quartz cuvette. For further characterization of the MAAs, freeze-dried samples were analyzed by high-performance liquid chromatography (HPLC). These samples (i.e., GF/F filters containing MAAs in seston and copepods) were extracted three times consecutively as described above. In the case of copepods, two replicates of 30 individuals each were extracted. The extracts from the three extractions were pooled and subsequently evaporated to dryness under vacuum in 2-ml eppendorf microcentrifugation tubes, using a SpeedVac concentrator (Savant) at 45°C. Afterwards, MAA analysis, identification, and quantification was as described in Tartarotti et al. (2001), except that a Phenomenex C-8 column (5-μm pore size, 4.6 mm i.d. × 25 cm) protected with a Brownlee RP-8 guard column and a diode array detector (Dionex) were used. Samples were run with a mobile phase of 0.1% acetic acid in 25% or 55% aqueous MeOH (v/v). Concentrations of MAAs were normalized to the dry weight of zooplankton, which was estimated by the relationship between body length and dry weight calculated for this species (Paggi 1983).

The activity of DNA photolyase was assessed by measuring the light-dependent loss of thymine containing pyrimidine dimers from UV-irradiated DNA. DNA was obtained from *Escherichia coli* B3 grown in a medium supplemented with [methyl-^{3}H]-thymidine (Johnson 1994). After extraction, the radiolabeled DNA was exposed to UVC (254 nm, 8.4 kJ m^{-2}) and used as substrate. The organisms under study were suspended in 2×10^{-2} M tris-(hydroxymethyl)-aminomethane/HCl pH 7.4, 10^{-1} M NaCl, 10^{-3} M ethylenediamine tetraacetic acid, 10^{-2} M dithiothreitol, and 10^{-3} M phenylmethylsulfonylfluoride and disrupted by ultrasonic treatment; debris was eliminated by centrifugation. Substrate was added to the extracts, and the mixtures were exposed to the radiation emitted by a Philips TDL 18W/08 tube and a Narva LT18W/010 SS tube at room temperature. To measure the content of pyrimidine dimers during incubation, DNA was obtained from the reaction mixtures by phenol extraction and ethanol precipitation, hydrolyzed as described by Carrier and Setlow (1971) and analyzed by reversed-phase HPLC following Niggli and Cerutti (1982). The enzyme activity was calculated from the data according to the method described by Cook and Worthy (1972).

The tolerance of *B. poppei* to artificial UV radiation was assessed using two experimental designs: dose response at a set end point and time-to-death (Newman 1995). The copepods were collected from the lakes within 72 h prior to the experiments and kept in the dark at 4°C on unfiltered lake water. The experiments were run using water from the original lakes containing natural seston as food.

For the assays that measured dose response at a set end point, the experimental unit consisted of 30 individuals that were placed in 5-cm-diameter petri dishes covered with quartz lids. The dishes were placed on a turntable to obtain a uniform dose inside a thermally isolated box between 1 and 5°C. The UV source, located at 26 cm above the turntable, was a Spectroline XX15-B fluorescent lamp (Spectronics Corp.) covered with a new sheet of cellulose diacetate to remove wavelengths shorter than ~295 nm (for description of the spectrum, *see* Zagarese et al. 1997). Four UV irradiance treatments (93, 76, 57, and 20% of full exposure [=60.2 μW cm^{-2}]) and a dark control were assayed on each experiment. The different irradiance treatments were obtained by covering the dishes with a variable number of layers of neutral-density filters (plastic window screen). Each treatment was run in three replicates. Initially, three different exposure times (6, 10, and 14 h) were assayed to obtain a wide range of mortality rates, but as we gained experience with this species, an appropriate exposure duration could be chosen with fewer trials. The total number of experiments and their exposure duration were as follows: Lake Boeckella, 1 × 6 h (i.e., one experiment of 6 h exposure) and 2 × 10 h; Lake Chico, 1 × 6 h, 1 × 10 h, and 1 × 14 h; Lake Esperanza, 1 × 10 h; and Lake Flora, 1 × 14 h. However in Fig. 2 and the statistical analysis, all results for the same lake were combined because exposure duration did not significantly affect the mortality versus dose response (multiple linear regression using logit-transformed mortality as dependent variable, UVB dose as a continuous independent variable, and exposure duration as a categorical independent variable). The copepods were kept in the dark until 24 h after the beginning of UV exposure (i.e., 18, 14, or 10 h depend-

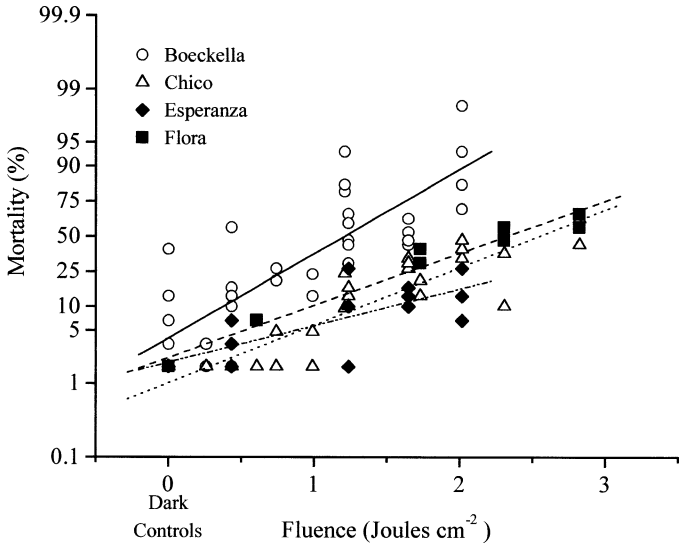

Fig. 2. Mortality versus dose curves for populations of *B. poppei* from different lakes exposed to UVB in the absence of recovery radiation. Note the logit scale of the *y*-axis. The lines were fitted by linear regression of logit-transformed mortality versus UVB fluence.

ing on UV exposure duration). During the following 24 h, the copepods were exposed to a 14 : 10 light : dark (LD) photoperiod. The number of survivors was counted after 48 h from the start of exposure.

To assess the potential effect of photorecovery, similar experiments were run in which the copepods were exposed simultaneously to UVR and photoreactivating (visible) light. In all cases, irradiation with visible light was done in a 14 : 10 LD photoperiod, starting at the same time as the UV exposure. The white light source (YZEL TL301411 W) irradiance was 1.094×10^{-3} μE cm^{-2} s^{-1}. For additional details of the experimental protocol, *see* Zagarese et al. (1997). The total number of experiments and their exposure duration were as follows: Lake Boeckella, 1 × 10 h (i.e., one experiment of 10 h exposure); Lake Chico, 1 × 10 h and 1 × 14 h; and Lake Flora, 1 × 14 h. The dose modification factor (DMF) was calculated as the quotient between the LD_{50} obtained in experiments in the presence and absence of recovery radiation. The LD_{50} values were calculated by linear regression of logit-transformed mortality rates versus UVB dose.

Two different sets of time-to-death experiments were performed. The first used an artificial source of UVB radiation, whereas the second used natural solar radiation. The experiment using artificial radiation started on 14 March 2000. It was run in a thermally isolated box between 1 and 5°C. The radiation sources and experimental conditions were the same as described above. The cellulose diacetate sheet was replaced daily to avoid photodegradation. The copepods were collected from Lakes Chico, Boeckella, and Flora 2 d before the beginning of the experiment. Each experimental unit consisted of 30 copepods placed in a petri dish (5 cm diameter), which was covered with a quartz lid. The animals were exposed daily to UV irradiance (55.8 μW cm^{-2}) and photoreactivating light (irradiance = 1.094×10^{-3} μE cm^{-2} s^{-1}) for a period of 6 h. Exposure to photoreactivating light continued for an additional period of 8 h after UV exposure to extend the period of potential photorepair beyond the finalization of UV exposure. Two different treatments were assayed for each lake: (1) UV-exposed (covered with quartz lids) and (2) dark controls (covered with aluminum foil). Each combination (lake × treatment) had three replicates. The number of survivors was recorded daily, immediately after UV irradiation. Daily doses were calculated from measurements of the lamp made with the IL-1700 radiometer.

The experiment using natural radiation was performed outdoors with individuals from Lakes Boeckella, Chico, and Esperanza. Thirty individuals were placed in quartz tubes (20 cm long, 1.7 cm diameter). One end of the tubes was closed with a silicon stopper, and the other end was covered with a 20-μm mesh gauze to allow diffusion of oxygen. Two treatments were run for each lake: (1) a full solar spectrum (quartz) and (2) a no-UV control (i.e., solar spectrum without the UV component, covered with UV-opaque acrylic with <1% transmittance below 374 nm). Each combination (lake × treatment) has four replicates. A rectangular tank (1 × 0.6 × 0.4 m), whose walls were thermally isolated with a 15-cm layer of Styrofoam, was used as a water bath to incubate the tubes. The tank, filled with unfiltered water from Lake Boeckella, had an east–west alignment. In order to maximize solar exposure, the tubes were arranged just below the surface on the south side. The experiment started on 1 March 2000. The organisms were exposed daily during daytime hours and covered with a thermal isolating lid at night to avoid freezing. On few occasions of extremely cold temperatures, the tubes had to be kept inside the laboratory. Survivors were recorded daily at dusk. The experiment ended 19 March. The daily doses were calculated from radiation measurements as above.

Results

The lakes included in this study are relatively small and shallow (max. depth: 7 m) and occur at low elevation (<100 m a.s.l). They are regularly exposed to very strong winds. For example, about 50% of the days during the study period had wind speeds above 70 km h^{-1}, and the maximum value during the study was 144 km h^{-1}. The lakes vary greatly in water transparency (K_d and a_d), Chl *a*, and DOC concentration (Table 1). The presence of MAAs in seston samples was confirmed in all the lakes assayed.

The copepod *B. poppei* occurred only in five out of the seven surveyed lakes (Boeckella, Chico, Encantado, Esperanza, and Flora). This species was highly tolerant to UVR exposure, as indicated by the LD_{50} values obtained for acute exposure to artificial UVR in the absence of photorecovery radiation (range: 1.18–2.47 J cm^{-2}), and in the presence of recovery radiation (range: 2.2–2.78 J cm^{-2}, Table 2). The individuals from Lake Boeckella exhibited the lowest tolerance to UVR exposure. Such a trend was consistent in the set-end-point exposure experiments (Fig. 2, Table 2; analysis of variance [ANOVA] $P < 0.001$), in the time-to-death experiments with artificial UVB radiation (Fig. 3, Table 2; repeated measures ANOVA $P < 0.001$), and in the time-to-

Table 2. Mean (SE) values of body length, dry weight, MAA concentration and optical density (OD) of aqueous methanolic extracts, photolyase activity, lethal doses with and without photoenzymatic repair (PER), and dose modification factor (DMF) for populations of *B. poppei* from five Antarctic lakes.

	Boeckella	Chico	Encantado	Esperanza	Flora
Length (mm)	1.88(0.027)	1.81(0.035)	1.75(0.028)	1.69(0.029)	1.77(0.028)
Dry weight (μg)*	55.2	46.8	42.9	39.3	44.4
MAAs (% dry wt)	0.02(0.0004)	0.11(0.006)	na	0.05(0.0003)	na
OD mean 335–338 per 100 individuals	0.099(0.006)	0.26(0.007)	0.11	0.26	0.14
Photolyase activity (h^{-1})	b.d.l.	b.d.l.–0.24	b.d.l.	b.d.l.	b.d.l.
LD_{50} without PER (J cm^{-2})	1.18(0.07)	2.28(0.10)	na	2.47(0.35)	2.28(0.11)
LD_{50} with PER (J cm^{-2})	2.2(0.12)	2.78(0.16)	na	na	2.55(0.13)
DMF	1.86	1.24	na	na	1.11
Time-to-death (artificial radiation) LD_{50} (J cm^{-2})	2.4(0.04)	4.9(0.11)	na	na	4.3(0.04)

* Estimated from the weight versus length relationship (Paggi 1983). b.d.l., Below detection limit.

death experiment with natural radiation (Fig. 4; repeated measures ANOVA $P < 0.001$). Long-wave UVA (over 374 nm) or even visible light could have been partly responsible for the high mortality observed in the no-UV controls, particularly in the individuals from Lake Boeckella.

Overall, the presence of photoreactivating light resulted in a significant increase in survival (ANOVA $P < 0.001$). However, the DMF always ranged between 1.11 and 1.86. (Such values may be considered modest when compared to species with higher photoreactivation capabilities, which usually display DMF higher than 4.) In addition, the activity of the enzyme photolyase was low and could only be detected in one sample from Lake Chico (Table 2).

Spectrophotometric scans of methanolic extracts of copepods from the different lakes revealed the presence of UV-absorbing compounds with absorption peaks between 335 and 338 nm (Fig. 5). The optical density at peak was lowest in copepods from Lake Boeckella (Table 2). The HPLC analysis confirmed the presence of three MAAs, which were detected both in phytoplankton and copepod samples (porphyra-334, mycosporine glycine, and shinorine, Fig. 6). The highest concentration corresponded to porphyra-334. The concentration of total MAAs was also lowest in copepods from Lake Boeckella (Table 2).

Discussion

The tolerance to UVR has been estimated previously for a few other species of the genus *Boeckella* using the same radiation source and similar experimental protocols. From a comparison of LD_{50} estimates (Table 3), *Boeckella poppei* stands out as a highly tolerant species. For example, the LD_{50} values measured in the absence of photorecovery radiation rank among the highest of the genus. On the other hand, in the presence of photorecovery radiation, only *B. antiqua* displayed higher tolerance. Such results suggest that *B. poppei*

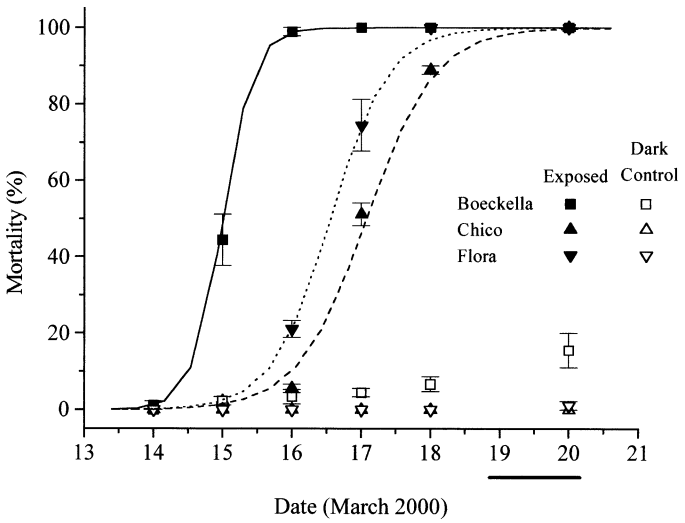

Fig. 3. Time-to-death experiment of copepods exposed simultaneously to artificial UVB and visible radiation. The horizontal line under the *x*-axis indicates days (19–20 March) on which the copepods were not exposed to artificial UVR. The lines were fitted by linear regression of logit-transformed mortality versus time.

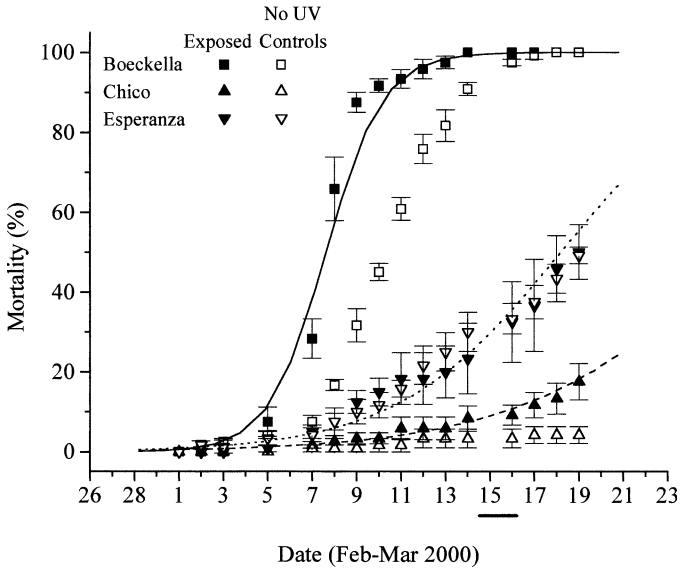

Fig. 4. Time-to-death experiment of copepods exposed to natural solar radiation. The horizontal line under the *x*-axis indicates days (15–16 March) on which the copepods were not exposed to natural UVR. The lines were fitted by linear regression of logit-transformed mortality versus time.

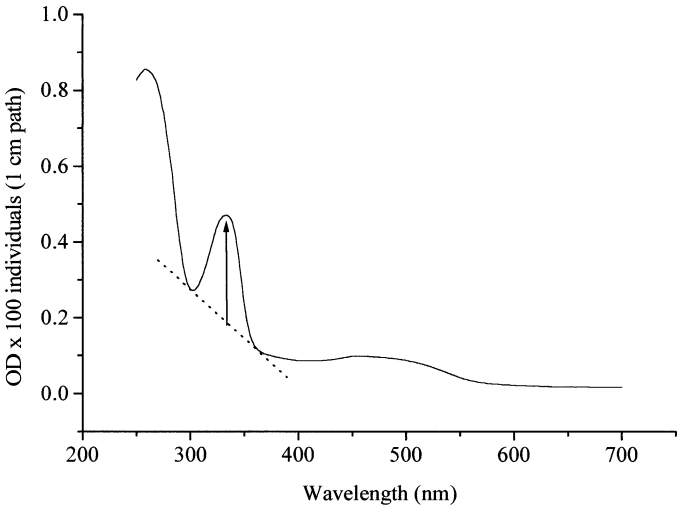

Fig. 5. Spectrophotometric scan of an aqueous methanolic (20%) extract of 100 individuals of *B. poppei* from Lake Chico. OD, optical density measured on a 1-cm cuvette. The height of the arrow corresponds to the estimated OD after subtraction of the baseline (dotted line).

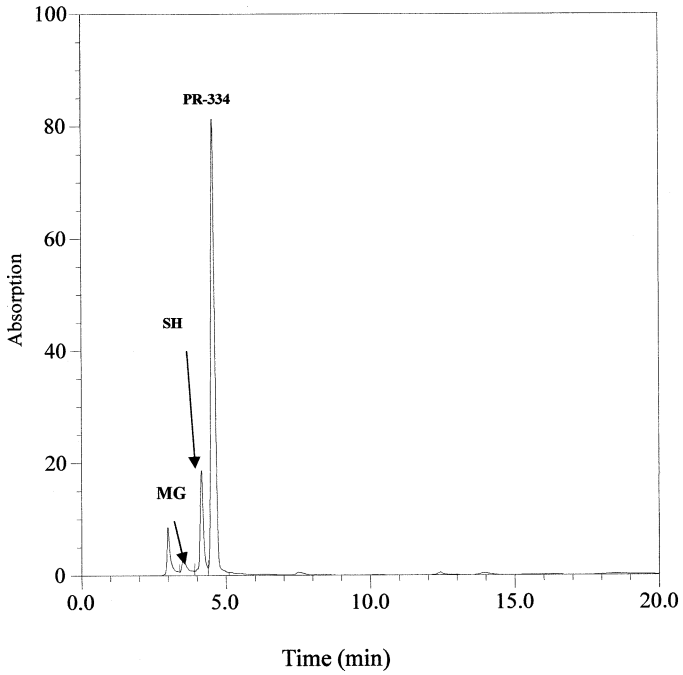

Fig. 6. HPLC chromatogram from an aqueous methanolic extract of *B. poppei* from Lake Chico. MG, mycosporine-glycine; SH, shinorine; PR-334, porphyra-334.

routinely experiences high doses of solar radiation in its natural habitats, indicating that the avoidance strategy may not be feasible, or at least not reliable. As already mentioned, this result was anticipated on the grounds of preliminary information suggesting that the Antarctic lakes included in this study were relatively transparent and shallow (Table 1) and highly exposed to the strong winds typical of those latitudes.

Interestingly, the potential for photorepair was minimal in all studied lakes. Although the presence of visible light resulted in statistically significant lower mortality (ANOVA, $P < 0.001$, data not shown), the increase in tolerance was relatively low (DMFs ranging from 1.11 to 1.86) when compared to species with strong photorecovery capabilities. These values are comparable to that of *B. gracilipes*, a species with almost negligible photorecovery capacity (Zagarese et al. 1997). In contrast, the DMFs for species with significant photorecovery are typically >4 (Siebeck and Böhm 1991; Zagarese et al. 1997). Moreover, although the presence of photolyase could be demonstrated in one of the samples, its activity ranged from low to undetectable (Table 2). Collectively, these two findings indicate a very low capacity for photorepair in *B. poppei.*

The presence of screening compounds in seston samples and copepods was confirmed in all the studied lakes. Furthermore, three MAAs could be identified by HPLC analysis (i.e., porphyra-334, shinorine, and mycosporine glycine). The results suggests a direct relationship between UVR tolerance and MAA concentration in *B. poppei*, since the individuals from Lake Boeckella showed lower tolerance and lower MAA concentration than their conspecifics from the other lakes (Table 2). A similar relationship has been observed in another species of this genus (*B. titicacae*) from Lake Titicaca (16°S, 68°W, 3,810 m a.s.l; Helbling et al. in press), suggesting that the accumulation of MAAs is a frequent response to UV exposure within the genus *Boeckella.*

Significant differences in the tolerance to UVR were detected between copepod populations from different lakes. As already mentioned, the copepods from Lake Boeckella were consistently less tolerant to UVR than the individuals from the other lakes (Figs. 2–4). This result may be the consequence of the higher UV attenuation and, presumably, lower UV exposure in Lake Boeckella (Table 1).

Out of the three possible strategies to cope with potentially damaging doses of UVR (i.e., avoidance, photoprotection, and repair), only the presence of photoprotection could be demonstrated in the populations of *B. poppei* from the studied Antarctic lakes. A priori, avoidance appeared as an

Table 3. Comparison of LD_{50} values with and without photoenzymatic repair (PER) for several *Boeckella* species.

Species	LD_{50} (J cm^{-2})		Habitat	Location	Source
	Without PER	With PER			
B. antiqua	1.8	>3.9	Shallow temporary ponds	Patagonian steppe	Zagarese et al. 1997
B. gibbosa	0.11	0.83	Clear mountain lakes	Northwestern Patagonia	Zagarese et al. 1997
B. gracilipes	0.13	0.13	Foothill lakes	Northwestern Patagonia	Zagarese et al. 1997
B. poppei	1.18–2.47	2.20–2.78	Antarctic lakes and ponds	Antarctic Peninsula	This study

unlikely strategy in these shallow and transparent environments regularly exposed to strong winds (up to 144 km h^{-1}). This prediction received further support from the exposure experiments that revealed high tolerance to UVR.

The low photorecovery capacity and low levels of photolyase activity indicates a rather limited use of repair mechanisms. An explanation for this may be related to the low temperatures prevailing in Antarctic lakes, which may limit the efficiency of enzymatic repair mechanisms. The same explanation has been offered to explain the low levels of photo enzymatic repair in bacterioplankton (Huot et al. 2000) and algae (Pakker et al. 2000) from cold environments. Photolyase binds to CPDs in DNA in a light-independent step. After absorbing a near-UV or visible photon, it splits the cyclobutane ring to restore the pyrimidines. Early studies demonstrated the influence of temperature on this process. The specific binding of yeast photolyase to damaged DNA was found to be impaired when assayed at 5°C instead of 37°C (Harm and Rupert 1970). Moreover, the rate of the light-dependent step for this enzyme decreases considerably when the reaction takes place at temperatures below −2°C (Harm 1969). These observations suggest that repair of DNA damage by photoreactivation would be limited at low temperatures. Similarly, a reduced efficiency of dimer removal by photoenzymatic repair was found at low temperatures in several biological systems, including a *phrB* mutant of *Escherichia coli* K12 (Dorrell et al. 1995), *Halobacterium cutirubrum* (Eker et al. 1991), cotyledons of *Cucumis sativus* (Takeuchi et al. 1996), fin and skin tissues of *Fundulus heteroclitus* (Malloy et al. 1997), and *Palmaria palmata*, for which photoreactivation effectiveness was almost negligible at 0°C compared to that found at 15 or 25°C (Pakker et al. 2000).

The results from this study indicate that the presence of photoprotective compounds may be the most efficient strategy in highly exposed habitats under low ambient temperatures. These results are consistent with the existence of a direct relationship between water transparency and MAA concentration, such as that found for populations of the copepod *Cyclops abyssorum* in mountain lakes of the Alps (Tartarotti et al. 2001). Moreover, they are also consistent with a direct relationship between MAA concentration and UVR tolerance, such as that found for several different kinds of organisms (Adams and Shick 1996), and particularly for another species of the genus *Boeckella* (i.e., *B. titicacae*; Helbling et al. 2002). Moreover, the postulated low efficiency of the enzymatic repair mechanisms at low temperatures may contribute to explain the inverse relationship between the concentration of photoprotective compounds and temperature (Byron 1982).

This study also confirms the tremendous plasticity of the genus *Boeckella* to cope with UVR under different environmental scenarios. For example, *B. poppei* from Antarctic lakes appear to rely almost exclusively on photoprotection, whereas *B. gibbosa* from high elevation lakes in the Andes depend fundamentally on photorepair (Zagarese et al. 1997) and *B. gracilipes*, which is highly sensitive, depend exclusively on avoidance by remaining deep in the water column of foothill lakes. Yet, other species, such as *Boeckella antiqua* (previously identified as *B. brevicaudata*), seem to take advantage of a combination of mechanisms (i.e., photoprotection and photorepair; Zagarese et al. 1997). The varied strategies to cope with UV radiation displayed by the genus *Boeckella* has probably been key to its success at colonizing a great variety of UV environments, ranging from large oligotrophic lakes to ephemeral pools, and from sea level to high-elevation habitats (Menu Marque et al. 2000).

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