Survivorship of *Cyclops abyssorum tatricus* (Cyclopoida, Copepoda) and *Boeckella gracilipes* (Calanoida, Copepoda) under ambient levels of solar UVB radiation in two high-mountain lakes

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**Abstract.** We performed in situ experiments during the summer of 1995 and 1996 to assess the potential effect of solar ultraviolet B (UVB) radiation (290–320 nm) on the survival of *Cyclops abyssorum tatricus* Kozminski and *Boeckella gracilipes* Daday. These species are numerically dominant within the crustacean zooplankton living in two high-mountain lakes, one located in the Austrian Alps [Gossenköllesee (GKS), 2417 m above sea level, maximum depth 9.9 m] and another in the Chilean Andes (Laguna Negra, 2700 m above sea level, maximum depth 320 m). The copepods were incubated in quartz tubes (1 l) or in quartz tubes wrapped with Mylar D® to exclude most of the UVB radiation. The organisms were exposed at 0.5 m depth for 10–72 h on cloudless days. Both lakes were very transparent to UVB and 10% of the surface radiation at the nominal wavelength of 305 nm was still present at 9.6 m in GKS and at 12.8 m in Laguna Negra. These species migrate vertically and have a maximum daytime distribution close to the bottom (*C.abyssorum tatricus*) or below 15 m depth (*B.gracilipes*). Both species were red, but the carotenoid concentration was higher in *C.abyssorum tatricus* than in *B.gracilipes* (6.5 and 2.3 µg mg–1 dry weight, respectively). UV-absorbing compounds with a maximum absorption at ~334 nm were also detected. *Cyclops abyssorum tatricus* was highly resistant to UVB radiation and no significant lethal effect was observed. *Boeckella gracilipes* had a mortality ~5 times higher in the treatment receiving full sunlight than in the Mylar treatment (3.2%) only when exposed for 70 h. The resistance of *B.gracilipes* was higher than that reported in the literature for the same species, suggesting the existence of intraspecific differences in UV sensitivity.

**Introduction**

During the last decade, an increase of solar ultraviolet B (UVB) radiation (290–320 nm) related to a stratospheric ozone depletion has been observed not only in Antarctica (Frederick and Snell, 1988), but also in northern latitudes, such as in the Swiss Alps (Blumthaler and Ambach, 1990) and Canada (Kerr and McElroy, 1993). Annual stratospheric ozone maxima have decreased between 1984 and 1993 by 5.8 and 3.2% in the southern and northern hemispheres, respectively (Bojkov and Fioletov, 1995). Therefore, many freshwater and marine ecosystems are potentially exposed to increased UVB fluxes (Williamson, 1995).

It has generally been assumed that aquatic ecosystems were well protected from the UVB radiation; however, several studies have revealed that it can reach considerable depths (Smith and Baker, 1979; Kirk, 1994; Morris et al., 1995; Sommaruga and Psenner, 1997). This is particularly true for lakes situated above the tree line where generally very low concentrations of chromophoric dissolved organic matter exist (Sommaruga and Psenner, 1997). In addition, lakes at high
elevations receive higher UVB radiation than those situated at sea level because of the natural increase with elevation (Blumthaler et al., 1992; Cabrera et al., 1995). The potential negative effects of solar UV radiation on planktonic organisms in alpine, highly UV-exposed habitats, were already suggested at the beginning of this century (Klausener, 1908; Brehm, 1938). Organisms living in these ecosystems, however, have probably developed different strategies to cope with high UV levels. For example, metazooplankton may have different ways to minimize the potential damage caused by solar radiation, such as behavioural avoidance (diel vertical migration), presence of photoprotective compounds (e.g. melanin, UV-absorbing compounds like mycosporine-like amino acids), anti-oxidants (carotenoid pigments), and effective DNA repair mechanisms (e.g. photoreactivation). On the other hand, the ecological and metabolic costs of these strategies may affect indirectly the survival, growth and reproduction (Zagarese and Williamson, 1994). Nevertheless, several studies have shown that artificial and solar UV radiation can directly impair the reproduction and survival of zooplankton (Siebeck, 1978; Karanas et al., 1979; Damkaer et al., 1980; Ringelberg et al., 1984; Siebeck and Böhm, 1991, 1994; Williamson et al., 1994; Zagarese et al., 1994, 1997a; Cabrera et al., 1997). However, the effect has been found to be species specific (Zagarese et al., 1997a) and dependent on the attenuation characteristics of UV radiation in the water column (Williamson et al., 1994; Zagarese et al., 1994).

In this study, we present results from in situ experiments designed to investigate the potential effect of ambient solar UVB radiation on the survival of two freshwater copepods, *Cyclops abyssorum tatricus* and *Boeckella gracilipes*, living in transparent high-mountain lakes of the Alps and Andes, respectively. The two lakes considered in this study differ not only in their species composition, but also in their possible depth refugia to avoid high solar radiation.

**Method**

**Study sites**

Gossenköllesee (GKS) is located in the Stubaier Alps, Tyrol, Austria (47°13′N, 11°01′E), at 2417 m above sea level (a.s.l.). In GKS, the only copepod species is *C.abyssorum tatricus* (Kozminski). Other species of crustacean zooplankton are *Chydorus sphaericus* (O.F. Müller) and *Alona affinis* Leydig. The most abundant rotifer species are *Polyarthra dolichoptera* Idelson and *Notholca squamula* (O.F. Müller) (Eppacher, 1968; Praptokardiyo, 1979; Tartarotti, 1997). Laguna Negra is situated in the Cordillera de los Andes Central, Chile (33°40′S, 70°06′W), at 2700 m a.s.l. In Laguna Negra, *B.gracilipes* Daday is the dominant crustacean species. Other zooplankton species described for this lake are *Boeckella gibbosa* Brehm, *Mesocyclops longisetus* (Thiébaud), *Microcyclops aniceps* (Richard), *Daphnia ambigua* Scourfield, *Daphnia pulex* De Geer, *Ceriodaphnia dubia* Richard and *Chydorus sphaericus* (O.F. Müller) (Araya and Zuñiga, 1985). The main rotifer species observed during the study were *Hexarthra fennica* (Levander), *Lepadella ovalis* (Müller) and *Lecane luna* (Müller). Relevant limnological information about the lakes is shown in Table I. In both lakes, predator fish have been introduced: *Salmo trutta* Linné f. *fario* in GKS and *Oncorhynchus mykiss* Walbaum in Laguna Negra.
Sampling

Experiments to assess the effect of solar UVB radiation on the survival of *C. abyssorum tatricus* and *B. gracilipes* were carried out from July to August 1995 and August 1996 in GKS, and during December 1995 and January 1996 (austral summer) in Laguna Negra. Other species were not included in this study because of their low abundance.

Samples were collected with a modified Schindler–Patalas sampler (5 l) in GKS or with a Van Dorn (5 l) sampler in Laguna Negra. The organisms were concentrated using a plankton sieve (45 µm mesh size). When the abundance of zooplankton was too low (July in GKS), samples were taken with a net (60 µm mesh size) making a vertical tow from near-bottom to surface.

Five and six experiments were carried out in GKS and Laguna Negra, respectively. Several exposure times ranging from 10 to 72 h were used during the experiments. For the short-term experiments (10–12 h), samples were collected at dawn, while for longer incubations (23–72 h) they were collected at dusk. In both lakes, samples were taken from 8 m depth, except in one experiment (Laguna Negra, 23 January) when they were collected from 25 m depth.

Experimental design

In all experiments, quartz tubes (1 l) with white silicone stoppers were used for solar radiation exposures. All experiments were performed on cloudless days. The quartz tubes were filled with the test species (GKS: $n_{\text{mean}} = 130$ individuals; Laguna Negra: $n_{\text{mean}} = 320$ individuals) and lake water (filtered through a plankton sieve of 45 µm mesh size) collected from the same depth and incubated *in situ* with the tubes held horizontally at 0.5 m depth. The following treatments were used in duplicates: (i) UVB + UVA + photosynthetically active radiation (PAR), i.e. full sunlight; (ii) UVA + PAR, using Mylar® D foil of 50 µm thickness (Du Pont de Nemours, 50% transmittance at 320 nm) to exclude the UVB; (iii) dark, i.e. tubes wrapped with three layers of aluminium foil. The transmittance characteristics of the Mylar® D

| Table I. Relevant limnological information for the study lakes. Chlorophyll $a$, dissolved organic carbon (DOC) and pH are the mean values for the epilimnion during summer |
|-----------------|-----------------|-----------------|
| Variable        | Gossenköllesee  | Laguna Negra    |
| Lake area (ha)  | 1.7             | 700             |
| Maximum depth (m)| 9.9             | 320             |
| Mean depth (m)  | 4.7             | 94.7            |
| Ice-cover duration (months) | 7–8             | Temporary       |
| Secchi disk depth (m) | 9.9             | 20              |
| Vertical attenuation coefficient for UVB (m$^{-1}$) | 0.24–0.32$^{a}$ | 0.18            |
| 10% attenuation depth for UVB (m) | 7.2–9.6$^{a}$  | 12.8            |
| Chlorophyll $a$ (µg l$^{-1}$) | <3              | <1              |
| DOC (µM C)      | 25              | n.d.            |
| pH              | 7.0             | 7.9             |

$^{a}$Values for Gossenköllesee are from Sommaruga and Psenner (1997). UVB corresponds to values from the 305 nm (Gossenköllesee) or 308 nm (Laguna Negra) channel of the underwater PUV sensor. n.d. = not determined.
foil used in this study are shown in Halac et al. (1997). Changes in the transmittance of the Mylar® foil were tested repeatedly in a double-beam spectrophotometer, and the foils were replaced if necessary. The tubes wrapped with aluminium foil were separated from the others to avoid possible reflection.

In GKS (August 1996), we performed one additional experiment to test whether delayed mortality in *C. abyssorum tatricus* may increase after exposure for 10 h when kept in darkness for another 20 h. The dark treatment served as a control for other possible effects, e.g. food shortage.

**Differentiation between live and dead individuals**

In order to distinguish live from dead copepods, we used a staining procedure with eosine (Eosine Y, Merck). This dye stains dead or partly damaged (e.g. broken antennula) copepods red. After incubation, the organisms were collected on a plankton sieve (45 µm mesh size) and transferred into 5 ml of filtered (10 µm mesh size) lake water. Then, 2.1 ml of eosine (6% vol/vol) were added and incubated for 10 min. The organisms were fixed with formaldehyde (18% final concentration) and collected on a 45 µm mesh sieve to remove the excess dye. Finally, the samples were preserved with formaldehyde (4% final concentration). The samples were counted immediately after staining because eosine is water soluble. Preliminary tests showed that our handling procedure did not produce any damage to the organisms.

The abundance of the different life stages (nauplii, copepodite CI–CV, adult female or male) and the number of eggs were enumerated under an inverted microscope (Leitz, Labovert) after sedimentation in an Utermöhl chamber.

**Vertical distribution of B. gracilipes and C. abyssorum tatricus**

In Laguna Negra, zooplankton were collected at local midday and midnight (10 January 1996) from surface to 24 m depth at 3 m intervals. In GKS, samples were taken during daytime from the surface to 9 m (1 m intervals) on 25 July 1995. The organisms were concentrated on a plankton sieve (45 µm mesh size) and preserved with formaldehyde (4% final concentration).

**Pigment analyses in zooplankton**

The carotenoid content was determined from copepods collected at the time of the incubation experiments. Replicates of ~80 cyclopoid copepods (copepodite CV and adult female, male) in GKS and 30 calanoid copepods (CV and adults from *B. gracilipes*) in Laguna Negra were extracted with 4 ml of absolute ethanol for 24 and 45 h, respectively (dark conditions, 20°C). The different time was necessary to obtain a complete extraction as checked by observation under the microscope. The optical density of the extract was measured with a double-beam spectrophotometer (Hitachi U-2000 or Shimadzu UV-160A) against an ethanol blank. Dry mass for the different stages was calculated from length–weight linear regressions established by Praptokardiyo (1979) for *C. abyssorum tatricus* and by
The carotenoid concentration was calculated according to the following formula:

$$\text{Carotenoid concentration (µg mg}^{-1} \text{ dry weight)} = \frac{(D \times V \times 10^4)}{(E \times W)}$$

where $D$ is the absorbance at peak, $V$ is volume of ethanol (ml), $E$ is the extinction coefficient set at 2500 and $W$ is the dry weight of the sample (mg) (Hairston, 1978; Hessen and Sørensen, 1990). To detect the presence of UV-absorbing compounds in *C.abyssorum tatricus* (all live stages), 100 individuals were carefully picked up and placed on a wet glass fibre GF/F filter (Whatman), then extracted with 13 ml aqueous methanol (90%) for 24 h (darkness, 4°C) and finally sonicated for 2 min on an ice bath. The optical density of the extract was measured with a double-beam spectrophotometer (Hitachi U-2000) against a methanol blank.

**UV and PAR measurements**

Surface and underwater radiation were measured during the incubations with a portable multichannel filter radiometer (PUV-500A/510A, Biospherical Instruments Inc.). The sensors have bandwidths ≤10 nm for the UV channels and measure at the following nominal wavelengths: 305/308, 320, 340 and 380 nm plus PAR (400–700 nm). The diffuse attenuation coefficient ($K_d$) was calculated from the slope of the linear regression of the natural logarithm of the downwelling irradiance ($E_d$) versus depth ($z$). The depth at which the irradiance was reduced to 10% ($Z_{10\%}$) of the value at the surface was calculated by dividing 2.3 by $K_d$.

**Results**

The maximum surface UVB$_{305\ nm}$ radiation during the study period was higher in Laguna Negra (0.14 W m$^{-2}$ nm$^{-1}$) than in GKS (0.071 W m$^{-2}$ nm$^{-1}$).

The initial percentage of dead individuals (natural mortality), as well as the mortality found in the dark controls, was negligible for both species. The results of all survivorship experiments are shown in Figure 1A and B for *C.abyssorum tatricus* and *B.gracilipes*, respectively. The mean percentage of dead individuals in *C.abyssorum tatricus* was <5%, and there was no statistically significant difference between the full-sunlight and the UVB-excluded treatments (Mann–Whitney test, $P > 0.05$). Even after 72 h of incubation, no significant mortality was observed. The experiment to test for the possibility of a delayed increase in mortality after exposure for 10 h gave negative results.

In Laguna Negra, exposures of *B.gracilipes* for 12, 23 and 48 h had no significant effect on the survival (mean percentage < 4%; Mann–Whitney test, $P > 0.05$). Only after an incubation period of 70 h was the mean mortality in the full-sunlight treatment 11.7% higher than in the treatment without UVB (Figure 1B).

The data of the vertical distribution of *B.gracilipes* in Laguna Negra showed a diel vertical migration pattern following the light regime (Figure 2). The abundance maximum at local midday was at 21 m depth (1% UVB attenuation depth
Fig. 1. Mortality of *Cabyssorum taticus* (A) and *B.gracilipes* (B) after exposure at 0.5 m depth to different solar spectral components versus exposure time in Gossenköllesee and Laguna Negra, respectively. Data are the mean percentage of mortality ± 1 SD.
at 25.5 m depth). In GKS, *C. abyssorum tatricus* remained close to the bottom (9.9 m depth) during daytime (Figure 2).

The pigment analyses showed an absorption peak at 474 nm characteristic of carotenoids in both copepod species and another absorption maximum at 334 nm. Figure 3 illustrates a typical result of an absorption spectrum for *C. abyssorum tatricus*. The carotenoid concentration in *B. gracilipes* (2.30 ± 0.43 SD µg mg⁻¹ dry weight) was lower than in *C. abyssorum tatricus* (6.5 ± 0.51 SD µg mg⁻¹ dry weight).

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**Fig. 2.** Vertical distribution of *B. gracilipes* at midday and midnight (10 January 1996) and of *C. abyssorum tatricus* during daytime (25 July 1995).

**Fig. 3.** Typical absorption spectrum of methanolic extracts made from *C. abyssorum tatricus*. 
Discussion

The lakes studied are among the most transparent to UVB radiation found in the literature (Kirk, 1994; Morris et al., 1995; Cabrera et al., 1997; Sommaruga and Psenner, 1997). For example, 10% of surface UVB was still present close to the lake bottom in GKS and at 12.8 m depth in Laguna Negra (Table I). Our data on the vertical distribution of *B.gracilipes* and *C.abyssorum tatricus* (Figure 2) indicate that the main daytime distribution of these species was close to or below the 10% attenuation depth of UVB radiation (305/308 nm). In our experiments, we transferred the copepods from close to or below the 10% UVB attenuation depth to the water surface. Consequently, the solar UV dose naturally experienced by these organisms was substantially increased. Even under this situation of forced UV exposure, we did not detect a significant lethal effect of UVB radiation on *C.abyssorum tatricus*. In the case of *B.gracilipes*, the mortality increased to 14.9% only when exposed for ~3 days to full sunlight; however, no significant effect was observed with shorter exposures (Figure 1B). Although 3 days can be considered a short period in terms of the population development or life span, most of the population is never exposed to such high UV doses under natural conditions. Moreover, results from a long-term (48 days) enclosure experiment at Laguna Negra suggest that the survivorship of *B.gracilipes* was not affected by UVB radiation (Cabrera et al., 1997). Nevertheless, it is worth noting that our study considered only lethal effects and therefore we cannot discard the possibility that sublethal effects may take place even at deeper layers in such transparent lakes like Laguna Negra.

Our results for *B.gracilipes* differ from previous investigations with the same species that suggested a high sensitivity not only to UVB, but also to UVA radiation (Zagarese et al., 1997a). For example, after an exposure to full sunlight at 0.5 m depth for 3 days, these authors observed a mortality of 79.1%. A direct comparison with our results is difficult because no information on the spectral characteristics of the solar radiation at the experimental sites is available. This is crucial because processes like, for example, DNA damage are strongly wavelength dependent. Nevertheless, one possible explanation for these contrasting results is that *B.gracilipes* in the study by Zagarese et al. (1997a) was collected from a lake located at 700 m a.s.l. with much lower transparency to UVB radiation ($z_{1%305\ nm} = 1.27$ m), but exposed in a lake of higher transparency ($z_{1%305\ nm} = 6.4$ m) and situated at 1700 m a.s.l. The combination of the higher surface UVB radiation due to the altitude effect and the lower underwater UV attenuation may have caused a higher impact on this species than it would usually experience in its original habitat. However, even when exposed in its original environment (3 days, 0.4 m depth), the mortality was high (62%) in the treatment exposed to full sunlight (Zagarese et al., 1998).

Another reason for the different sensitivity of *B.gracilipes* in these two regions may rely on the different pigmentation. In Laguna Negra, the copepods were slightly red, whereas those of the Nahuelhuapi lakes (41°S) area were translucent (Zagarese et al., 1997a). Pigmented species are considered to be less sensitive to solar radiation than unpigmented species (Hairston, 1978; Byron, 1982;
Ringelberg et al., 1984; Siebeck and Böhm, 1994). Hairston (1979) found higher concentrations of carotenoids in red morphs compared to pale morphs and a nearly 10-fold difference in the weight-specific carotenoid content of *Diaptomus nevadensis* populations from two different lakes. Although carotenoid pigments are not UV filters, they are important in protecting organisms against the potential damage caused by photo-oxidative stress (Krinsky, 1979). The occurrence of UV-absorbing compounds in *B. gracilipes* (maximum absorption at 334 nm) in Laguna Negra is not described yet for the populations occurring in the Nahuelhuapi lakes area.

The *B. gracilipes* population from Lake Ezquerra (41°S) studied by Zagarese et al. (1997a) lacked a mechanism for photorepair. Whether *B. gracilipes* from Laguna Negra is able to photorepair potential DNA damage, as observed in different zooplankton species (Siebeck, 1978; Siebeck and Böhm, 1991; Zagarese et al., 1997a), is not known. As photoreactivation was shown to be mostly responsible for the UVB resistance in *Boeckella gibbosa* (Zagarese et al., 1997b), differences in the ability to photorepair may be associated with interspecific UV sensitivity. Whereas *B. gracilipes* is described as a common pelagic zooplankton species for several high-mountain lakes of Chile (Villalobos and Zuñiga, 1991), *B. gibbosa* is dominating the zooplankton communities in lakes and ponds of higher altitude and latitude in Patagonia (Zagarese et al., 1997b). No occurrence of *B. gracilipes* was observed in high-elevation lakes in this region (B. Tartarotti, unpublished data).

There is no previous information on the UV sensitivity of *C. abyssorum tatricus*; however, our results indicate that this species is very tolerant to the high solar UV radiation found in clear-water alpine lakes. Resistance against UVB may partially explain its wide distribution, ranging from shallow pools to deep lakes (Gliwicz, 1986; Schaber, 1988; Tartarotti, 1997). Characteristically, this species has a vivid red pigmentation and the carotenoid concentration was almost three times higher than in *B. gracilipes*, but similar to that of *Diacyclops bicuspidatus* (6 µg l⁻¹ mg⁻¹ DW), *Heterococe saliens* (7.96 µg mg⁻¹ DW) and the red morphotype of *Diaptomus kenai* (7.49 µg mg⁻¹ DW), all living in alpine lakes (Hairston, 1978; Hessen and Sørensen, 1990; Hessen, 1992). In addition, we detected the existence of UV-absorbing compounds in *C. abyssorum tatricus* with a maximum of absorption (λ_max) at 334 nm (Figure 3). Recently, Sommaruga and Garcia-Pichel (1999) identified these mycosporine-like compounds as shinorine (λ_max = 334 nm), asterina-330 (λ_max = 330 nm), mycosporine-glycine (λ_max = 310 nm) and another one tentatively identified as palythine (λ_max = 320 nm).

Although *C. abyssorum tatricus* was found to be UV resistant, our data and those from earlier studies (Eppacher, 1968) show that this species avoids the surface waters during the day, having a maximum occurrence close to the bottom (9 m depth). Several authors have suggested that diel vertical migration is a natural response to UVB (Damkaer, 1982; Hessen 1992, 1994) as migration is also observed in habitats lacking predators. However, Zagarese et al. (1997b) suggested that UVB is unlikely to be responsible for the deep vertical distribution during daytime. In GKS, Gliwicz (1986) indicated that fish predation was the major factor explaining the existence of a diel vertical migration pattern in this species.
In the present study, *C.abyssorum tatricus* and *B.gracilipes* experienced a 10- to 100-fold increase in UVB radiation compared to the levels found at their maximum daytime distribution. Our data indicate that both species are relatively resistant when exposed to high ambient UVB levels.

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