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## Copepod mortality induced by fluctuating levels of natural ultraviolet radiation simulating vertical water mixing

**Abstract**—The effect of fluctuating ultraviolet radiation levels on the copepod *Boeckella gracilipes* was investigated in Lake Escondido (Patagonia, Argentina). The animals were incubated either at fixed depths or rotating in situ plankton wheels of different diameters. The observed mortality was significantly higher in rotating treatments. Static incubations can be used to predict the mortality of vertically moving *B. gracilipes*, providing that the doses of UVA and UVB are known. The results suggest that under moderate wind conditions, the plankton of shallow lakes are exposed to potentially damaging levels of solar radiation, even in relatively turbid waters.

Natural solar radiation is now recognized as a strong selective force in aquatic ecosystems. Although much of the recent work on UV photobiology has been stimulated by future prospects of increases in UVB intensities due to stratospheric ozone depletion, the accumulated evidence demonstrates that UV levels considered normal have significant impacts on natural communities (Williamson 1995).

Recent studies using newly developed submersible radiometers have identified dissolved organic carbon (DOC) concentration as the most important factor controlling the attenuation of ultraviolet radiation (UVR) in lakes (Scully and Lean 1994; Morris et al. 1995; Morris and Hargreaves 1997). It has also been suggested that changes in DOC concentration may be more important than stratospheric ozone depletion in regulating future changes in UVR in natural freshwater ecosystems (Schindler et al. 1996; Williamson et al. 1996).

The physical processes controlling UVR penetration in freshwaters are reasonably well understood (Kirk 1994). In addition, the use of biological dosimeters (Karentz and Lutze 1990; Regan et al. 1992; Kirk et al. 1994) and in situ incubations of planktonic organisms (Williamson et al. 1994; Zagarese et al. 1994) have helped in understanding the at-

tenuation of the potential damage with depth. However, damage assessment is complicated by the fact that planktonic organisms may experience tremendous fluctuations in the level of radiation because of their vertical displacements. (Helbling et al. 1994; Jeffrey et al. 1996). Wind stress is usually responsible for turbulent mixing in surface waters (Imboden and Wüest 1995). In Patagonia (Argentina), the mean wind speed during spring and summer is 32 km h<sup>-1</sup>, and strong winds (>43 km h<sup>-1</sup>) blow 41% of the days of the year (Baigún and Marinone 1995).

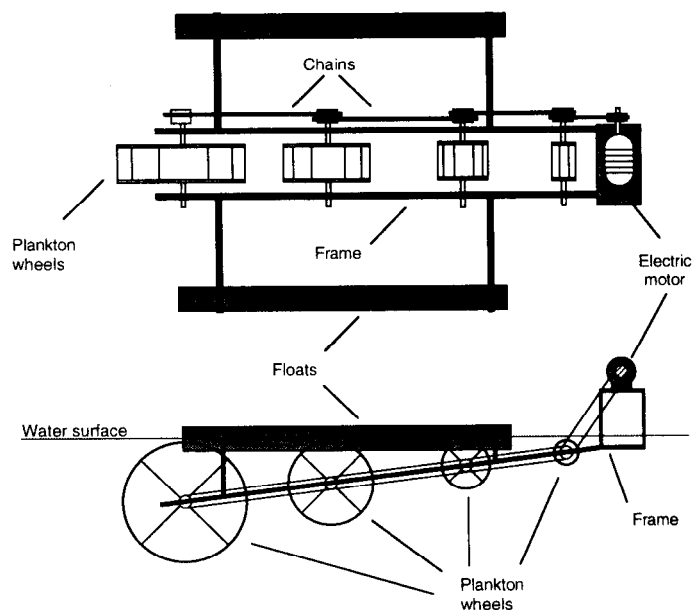


Fig. 1. Top view and side view of the incubation apparatus (see description in text).

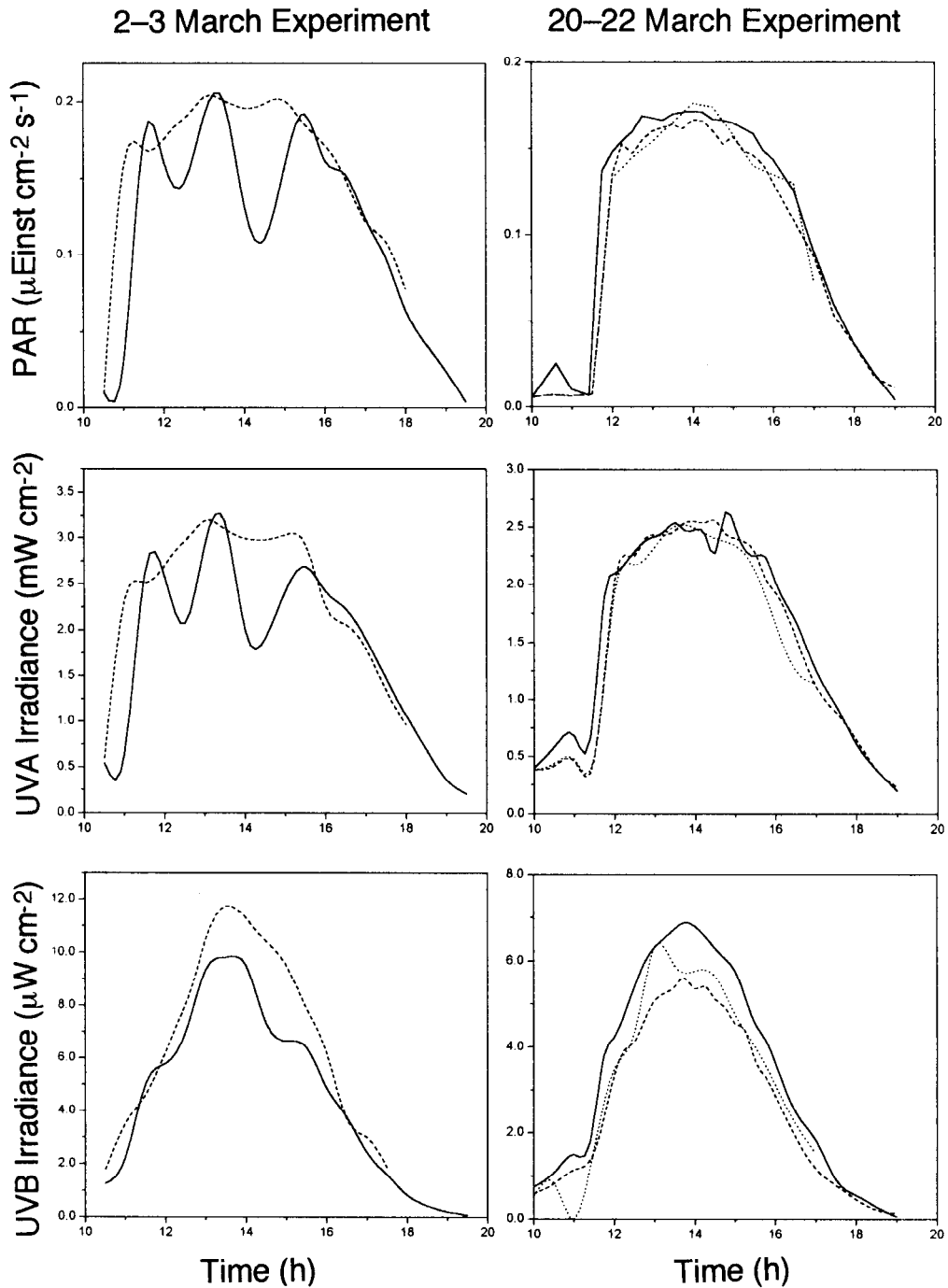


Fig. 2. Irradiance conditions during each experiment: —, first day; ---, second day; ·····, third day (20–22 March experiment only).

In this study, we compare the vulnerability to solar radiation of copepods that were experimentally forced to cycle near the surface to that of copepods incubated at fixed depths. Consider an animal rotating vertically around a second individual that remains at a fixed depth. Although their mean depths are the same when averaged over time, the radiation experienced by them differs in three important respects. (1) Because radiation attenuates exponentially with

depth, a vertically moving organism will be exposed to higher doses of any given wavelength. (2) Because of the inverse relationship between attenuation and wavelength (Morris et al. 1995), the spectral composition experienced by such an organism will be shifted toward shorter wavelengths. (3) While a static organism will experience a relatively constant irradiance, the organism that moves up and down will be exposed to pulses of radiation many times higher than the

mean, followed by periods of very low intensity. Unless reciprocity can be assumed (i.e. unless the effects depend only on the dose, regardless of the dose rate), the effects on static and moving organisms are likely to be different. A major reason for the frequent failure of reciprocity is the presence of repair mechanisms (Lesser et al. 1994), such as photo-reativation.

Experiments were performed in Lake Escondido, a slightly humic lake (DOC of  $2.66 \text{ g m}^{-3}$ ) in the Nahuel Huapi National Park. The relatively turbid water of Lake Escondido ( $K_d(305)$  of  $8.88 \text{ m}^{-1}$ ; Morris et al. 1995) provided the required attenuation of ultraviolet light within a convenient vertical scale. Whereas the radiation source and the attenuation pattern used in the experiments were natural, the vertical cycling of the zooplankton had to be simulated. The incubations were performed using an apparatus that holds four plankton wheels of different diameters (15, 32, 60, and 80 cm). The apparatus was designed to operate continuously in situ for several hours. The four wheels were moved by a 12-V DC motor powered by a standard car battery and transmission chains, so that their angular velocities were identical. Two floats placed at both sides of the structure maintained the system in the desired position, with the floatation line being adjusted very accurately by means of screws. The distance between the wheels as well as their sizes were calculated so that the surface of the water would be tangent to the upper edge of the wheels (Fig. 1). The wheels completed a cycle in about 2.25 min. The spatial and temporal scales of this motion pattern are within the range observed under turbulent water mixing induced by moderate winds (up to meters per minute; MacIntyre 1993; Imboden and Wüest 1995).

The species studied was *Boeckella gracilipes* Daday, a small herbivorous calanoid copepod, which is very common in Patagonia. Previous studies have shown that *B. gracilipes* had little if any potential for photoreactivation (Zagarese et al. 1997a). The lack of photoreactivation was expected to increase the chances of meeting the assumptions of reciprocity, therefore simplifying the interpretation of the results. Although *B. gracilipes* occurs in Lake Escondido, large numbers of *Bosmina longirostris* in the samples made the separation of individuals extremely time consuming. For the experiments we used individuals collected from nearby Lake Nahuel Huapi. The copepods were incubated in 40-ml quartz tubes and closed with silicone stoppers (20 adults or late copepods per tube), the tubes having been previously filled with filtered ( $45 \mu\text{m}$ ) lake water and additions of *Chlamydomonas reinhardtii*. Four replicate tubes of each treatment were incubated either static or rotating in the plankton wheels. An additional treatment, fully wrapped in aluminum foil, served as dark control. The static treatments were suspended at the same depths as the axes of the wheels (i.e. 7.5, 16, 30, and 40 cm). The wheels were maintained rotating continuously during the day, but their motion was suspended during the night.

The first experiment lasted 2 d (2–3 March 1996). The copepods were collected and sorted into the quartz tubes the previous day. In order to compensate for lower UVB irradiance later in the season (Fig. 2), the second experiment was run for 3 d (22–24 March 1996). For this experiment,

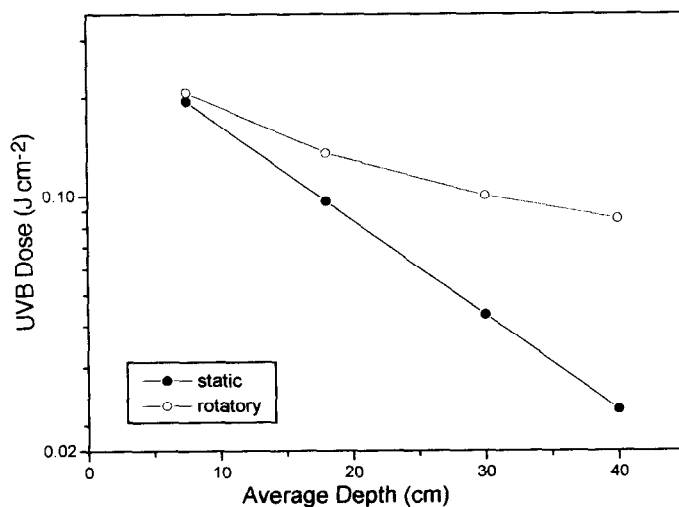


Fig. 3. UVB doses for the 20–22 March experiment. The rotating treatments received higher doses and the difference increased with depth.

the copepods were collected 2 d before the experiment and sorted into the quartz tubes the day before the experiment.

Ultraviolet and photosynthetically active radiation (PAR) irradiances were recorded with an IL-1700 portable radiometer (International Light) equipped with UVA and UVB detectors centered at 295 and 356 nm, respectively, and a PAR detector with a flat response filter between 400 and 700 nm (see Kirk et al. 1994). Ground-level radiation was measured at least every 30 min., and underwater measurements were taken several times during the experiments. By integrating the surface irradiance  $I_{UVA}$  and  $I_{UVB}$  (Fig. 2) weighted by the corresponding attenuation during the experiments, we obtained the total UVA and UVB doses  $D$  as a function of the mean depth by equations

$$D_{UVX}(\bar{z}) = \int_t I_{UVX}(t) \exp(-K_d^{UVX} \bar{z}) dt$$

for the static treatments, and

$$D_{UVX}(\bar{z}) = \int_t I_{UVX}(t) \exp\{-K_d^{UVX} \bar{z} [1 + \cos(\omega t)]\} dt$$

for the rotating treatments. The index "X" stands for the different UV regions A or B, and  $\omega$  is the angular velocity of the wheels ( $\sim 0.047 \text{ s}^{-1}$ ). Fig. 3 shows the results of the calculated UVB dose for the 20–22 March experiment.

In the first experiment the mortality in the controls was 7.5%, while in the treatments the mortality ranged between 11 and 94%. Except for the tubes incubated at 7.5 cm, the mortality was higher in the rotating treatments than in the static treatments at the same average depth (Fig. 4A). The difference (after excluding the tubes incubated at 7.5 cm) was significant (two-way ANOVA,  $N = 21$ ,  $p_{\text{depth}} < 0.01$ ;  $p_{\text{motion}} < 0.01$ ;  $p_{\text{depth} \times \text{motion}} > 0.1$ ). In the second experiment, the mortality in the dark controls was 21% and ranged between 62 and 100% in the other treatments (Fig. 4B). As in

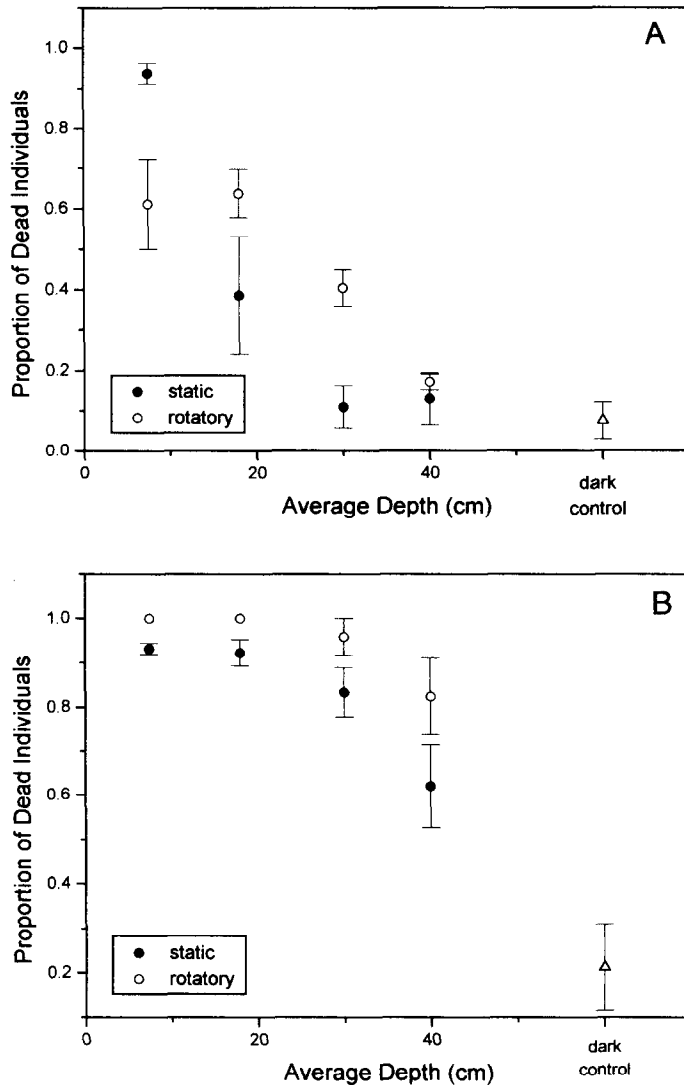


Fig. 4. Mortality of *B. gracilipes* as a function of the average incubation depth, 2-3 March (A) and 20-22 March (B).

the first experiment, the mortality was higher in the rotating treatments than in the static treatments at the same average depth (two-way ANOVA,  $N = 28$ ,  $p_{\text{depth}} < 0.01$ ;  $p_{\text{motion}} < 0.01$ ;  $p_{\text{depth} \times \text{motion}} > 0.1$ ).

We were also interested in knowing if static incubations could be used to predict the mortality rate of vertically moving copepods. Because static and rotating treatments received different combinations of UVA and UVB radiation (Fig. 5), and given that both portions of the solar spectrum are known to be damaging to *B. gracilipes* (Zagarese et al. 1997a), our approach was to use the information from the static treatments to develop a model that could be used to estimate the mortality rate ( $d$ ) for any given combination of UVA and UVB doses. The model's predictions could then be compared to the mortality observed in the rotating treatments. The model is based on the logistic equation

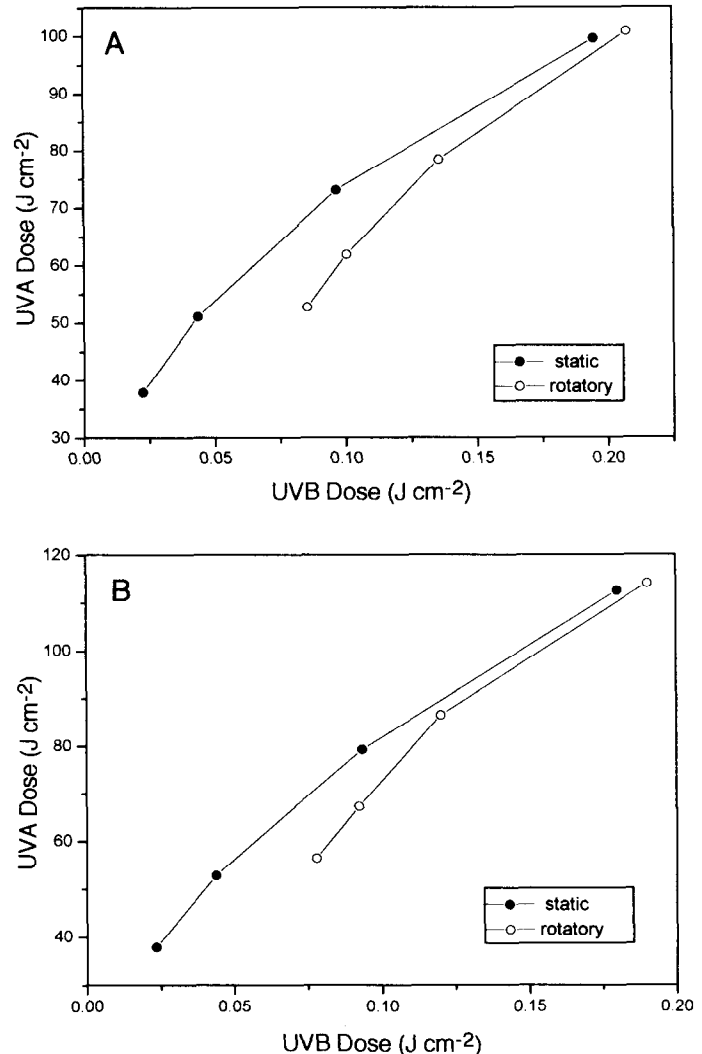


Fig. 5. UVA dose as a function of the UVB dose, showing the difference in spectral composition between different treatments, 2-3 March (A) and 20-22 March (B).

$$d = \frac{\exp(k_1 i_1 + k_2 i_2 + a\text{UVA} + b\text{UVB})}{1 + \exp(k_1 i_1 + k_2 i_2 + a\text{UVA} + b\text{UVB})}$$

where  $k_1$  and  $k_2$  are the background mortalities for the Exp. 1 and 2, respectively;  $i_1$  takes the value of 1 if the data come from experiment 1, and 0 otherwise;  $i_2$  does the opposite; and  $a$  and  $b$  are the coefficients for the UVA and UVB doses, respectively. We fit the model using the Systat NONLIN procedure, yielding parameter estimates  $k_1 = -4.5903$ ;  $k_2 = -1.2317$ ;  $a = 0.0357 \text{ cm}^2 \text{ J}^{-1}$ ; and  $b = 16.8507 \text{ cm}^2 \text{ J}^{-1}$  ( $r^2 = 0.99$ ). The products of coefficient by magnitude were similar for UVA and UVB. The model was subsequently used to estimate the expected dead rates in the rotating treatments. The agreement between expected and observed mortality rates was remarkably good ( $r^2 = 0.98$ ; Fig. 6).

The results show that the mortality in the rotating treatments can be predicted very accurately using the mortality information from the static incubations in combination with

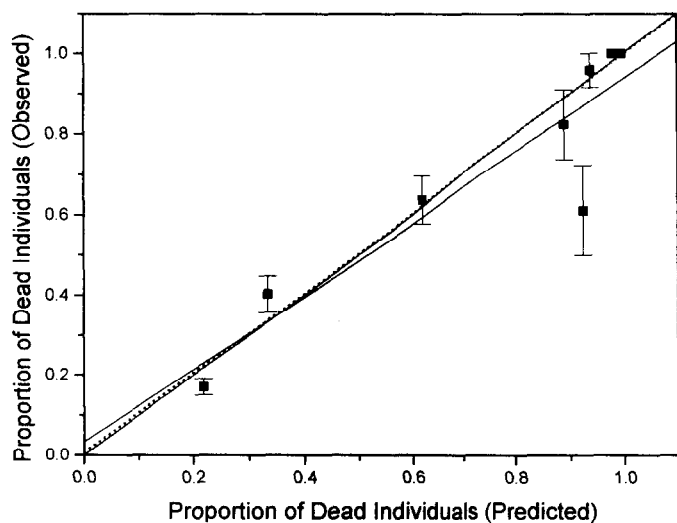


Fig. 6. Observed vs. predicted mortality for the rotatory treatments. —, identity; ---, linear fit including all datapoints; ·····, linear fit excluding the datum corresponding to the 2–3 March experiment at 7.5-cm average depth.

radiation measurements in two regions of the ultraviolet spectrum. Because not only the dose, but also the dose rate and the spectral composition of the radiation experienced by the rotating and static treatments were different, the agreement between observed and predicted mortality supports two important assumptions implicit to our approach. First, within the range of experimental conditions, the law of reciprocity holds for *B. gracilipes*. Actually, by choosing a species with virtually no photoreactivation capacity (Zagarese et al. 1997a), we attempted to meet the assumptions of the law of reciprocity. Second, radiation measurements in two regions of the ultraviolet range appear to capture a great deal of information about the solar spectrum in the ultraviolet range. Similarly, Kirk et al. (1994) and Dahlback (1996) have shown that it is possible to generate spectra similar to those measured by spectroradiometers using data from narrow-band radiometers.

Comparing the cruise velocity of crustacean zooplankton ( $\sim 12\text{--}24\text{ cm min}^{-1}$  for a 1-mm copepod; Peters et al. 1994) and the turbulent velocity induced by moderate winds ( $\text{m min}^{-1}$ , MacIntyre 1993; Imboden and Wüest 1995), we may conclude that small organisms could hardly avoid being cycled in the water column of a shallow lake. Moreover, the energy required for swimming may represent a significant proportion of an organism's energy expenditure (Peters et al. 1994), while the cost of maintaining mechanisms to counteract the effects of UV radiation appears to be low (Zagarese et al. 1997b). Our results confirmed that the potential damage experienced by vertically moving organisms is higher than that experienced by static organisms at the same average depth. Therefore, the effects of solar radiation in shallow lakes may be significant, even if the water is relatively turbid. This could explain the frequent occurrence of pigmented, UV-resistant copepods in highly productive or

humic ponds (Luecke and O'Brien 1981; Byron 1982; Zagarese et al. 1997a).

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