UV damage in shallow lakes: the implications of water mixing

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Abstract. The plankton of shallow lakes is expected to experience increasing exposure to ultraviolet radiation (UVR) within the next decades. In addition to stratospheric ozone depletion, decreases in dissolved organic carbon (DOC), caused by climate warming or acidification, will result in greater UVR penetration in many lakes. Circulation patterns induced by moderate winds force the plankton of shallow lakes to be cycled through the whole water column. This translates into higher doses and fluctuating dosage rates. Here, we report the results of a series of 'in situ' UV exposure experiments in which two zooplankton crustaceans (one capable of photorecovery and the other not) were incubated either at fixed depths or under simulated mixing. Our main goal was to assess the extent to which the results from static incubations could be extrapolated to vertically moving zooplankton. Static incubations accurately predicted the survival of moving individuals for the species without photorecovery, but failed to do so for the species capable of photorecovery. The fluctuations in dosage rate for high doses tended to increase the survival of the species with photorecovery. We contend that neglecting the effects of vertical mixing may result in a biased perception of UVR effects in shallow lakes.

Introduction

The plankton of shallow lakes are expected to experience increasing exposure to ultraviolet radiation (UVR) within the next decades. In addition to stratospheric ozone depletion (Lubin and Jensen, 1995), decreases in dissolved organic carbon (DOC) in lakes, caused by climate warming or acidification, will result in greater UVR penetration in many lakes (Schindler et al., 1996; Yan et al., 1996). The downward irradiance of UVR in lakes is mainly controlled by the concentration of DOC (Scully and Lean, 1994; Morris et al., 1995). However, in a well-mixed water column, the average dosage rate experienced by free-moving organisms is a function not only of DOC, but also of mixing depth (= lake depth for shallow lakes). Moreover, the average dosage rate for well-mixed, moderately high DOC lakes is primarily determined by the lake depth, and is relatively insensitive to changes in DOC (Figure 1). The consequences of water mixing cannot be overlooked in regions, such as Patagonia (Southern Argentina), where the mean wind speed during spring and summer is well over 30 km h^{-1} (Baigún and Marinone, 1995).

Mixing causes planktonic organisms to be exposed to fluctuating radiation levels that may vary over a range of several orders of magnitude (Smith, 1989). Under the assumptions of reciprocity (Bunsen–Roscoe law), the organisms average out the fluctuations in dosage rate and, consequently, the effects can be reasonably modeled as a function of dose. However, reciprocity can hardly be
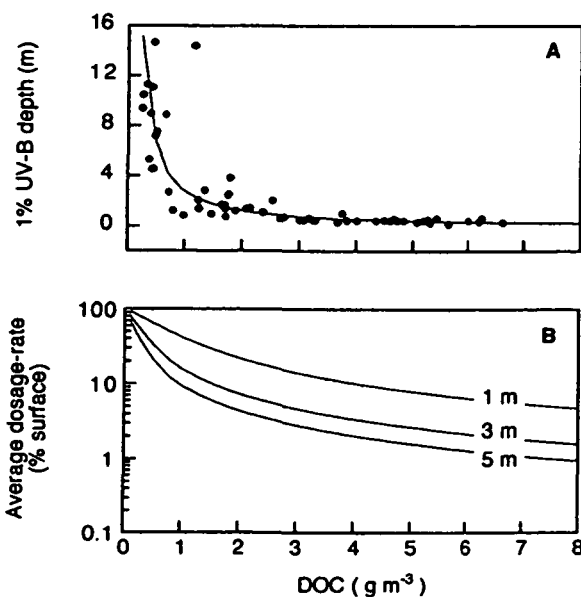
Fig. 1. (A) The relationship between the 1% UVB isopleth (λ 320 nm) and the concentration of DOC from 64 lake sites in North and South America. (B) The average dosage rate (ADR) experienced by moving organisms in a well-mixed water column as function of DOC for three different lake depths. Although at DOC concentrations higher than ~1 g m^{-3} the UVR is attenuated very rapidly, the average dosage rate experienced by planktonic organisms may be quite high in well-mixed shallow lakes. The lines correspond to the equation: ADR $= I_0 \times (1 - \exp(-K_d \times Z))/(K_d \times Z)$, where I_0 (= 100%) is the irradiance at the water surface, K_d is the diffuse extinction coefficient for λ = 320 nm and Z is the lake depth [data from Morris *et al.* (1995)].

claimed to be the rule in aquatic ecosystems (Cullen and Neale, 1994). The activity of repair processes has been shown to result in the failure of reciprocity (Lesser *et al.*, 1994). There is evidence that repair systems are induced after a period of exposure to UVR (Buma *et al.*, 1996). Although under the constant irradiance conditions of laboratory experiments a balance between damage and repair can eventually be established (Cullen and Lesser, 1991; Buma *et al.*, 1996), such a steady state may be delayed or even prevented under the fluctuating irradiance of natural conditions.

Here, we report the results of a series of '*in situ*' UV exposure experiments in which two zooplankton crustaceans were incubated either at fixed depths or under simulated mixing. Unlike previous studies with primary producers (Helbling *et al.*, 1994), the use of heterotrophs here should allow us to interpret the results in terms of damage and recovery without the interference of photosynthesis (Marra, 1978; Gallegos and Platt, 1982; Mallin and Paerl, 1992; Grobbelaar, 1994). The choice of the species was based on their potential for recovering from UV damage: photorecovery (i.e. enhanced survival mediated by the presence of visible radiation) is present in *Ceriodaphnia dubia*, but is absent in *Boeckella gracilipes* (Figure 2).
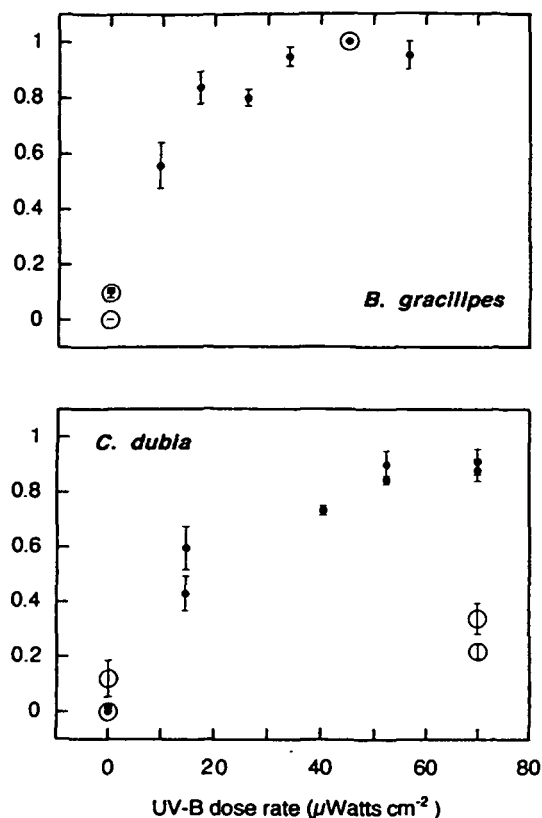
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Fig. 2. UVB-induced mortality in the presence (dark circles) and absence (large open circles) of visible light (recovery radiation). Top, *B.gracilipes*; bottom, *C.dubia* [data from Zagarese *et al.* (1997) and unpublished experiments].

In a previous study (Zagarese *et al.*, 1998), we found that the survival of static and vertically moving *B.gracilipes* individuals was virtually identical at comparable doses, suggesting that reciprocity was satisfied within the experimental conditions. On the other hand, for a species capable of photorecovery, such as *C.dubia*, we do not expect that reciprocity would hold. Our working hypothesis was that the departure from reciprocity would be larger at the high dosage rate end and, consequently, the high subsurface dosage rates experienced only by moving individuals would have a disproportionally high effect on mortality. In other words, at comparable doses, we expected the survival of moving *C.dubia* individuals to be lower than that of static individuals.

Method

The field experiments were performed in Lake Trébol (41°03'S, 71°31'W) during January 1997. Trébol is a small, oligotrophic (chlorophyll a = 0.9 g m^{-3}) lake in

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Nahuel Huapi National Park, Northwestern Patagonia, Argentina. Its slightly humic water (DOC = 1.7 g m^{-3}) provided the required attenuation of UVR within a convenient vertical scale. The incubation device (Figure 3) holds four vertical plankton wheels of different radii (7.5, 16, 30 and 40 cm). The four wheels are tangent to the water surface and complete a cycle in ~3–4 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). In addition to the four rotating treatments, four static treatments were placed in UVT acrylic racks suspended at the same depths as the wheels' axes. The use of wheels of different diameters allowed us to compare static and moving treatments across a wide range of UVR doses.

Twenty adult individuals of either species were placed in quartz tubes (40 ml) filled with filtered (GF/F) lake water plus additions of *Chlamydomonas reinhardtii* from a culture. The tubes were incubated either at fixed depth over the acrylic racks or rotating in the plankton wheels. An additional treatment, fully wrapped in aluminum foil, served as a dark control. Each treatment had three replicates. The experimentation period was 3–4 days. We ran three independent experiments with *C.dubia* and one experiment with *B.gracilipes*. The experiment
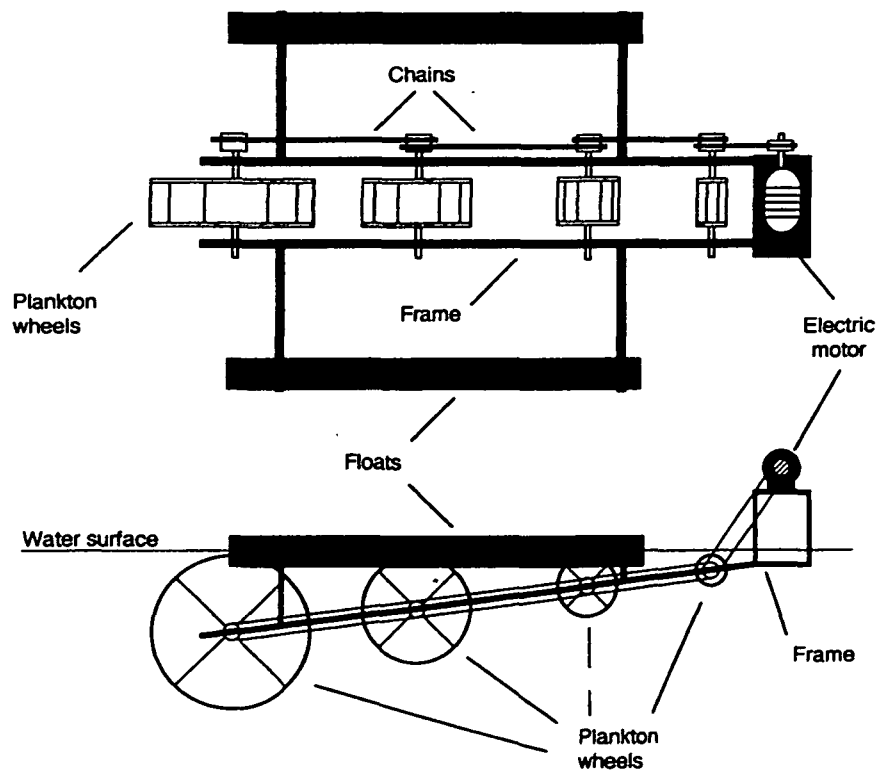

Fig. 3. Top view and side view of the incubation apparatus. (Redrawn from Zagarese *et al.* 1998)

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with *B.gracilipes* was mainly intended as a reference against which to compare the results obtained with *C.dubia*, but it also served to assess the degree of reproducibility of previous results (Zagarese *et al.*, 1998). Ground level radiation was measured every 20 min with an IL-1700 radiometer from International Light Inc. equipped with broad-band submersible detectors [SUD033, photosynthetically active radiation (PAR); SUD033, UVA; SUD240, UVB]. The mean attenuation coefficient for each radiation band was taken several times during the experimentation period. Admittedly, the attenuation coefficients calculated from measurements made with an IL-1700 are prone to errors due to a drift in the spectral composition with depth. Nevertheless, they still provide a reasonably good description of the radiation environment experienced by the organisms (Zagarese *et al.*, 1998). By integrating the surface irradiance of UVA (IUV-A) and UVB (IUV-B) (Figure 4) weighted by the corresponding attenuations (K_d) (Figure 5) over the duration of the experiments, we obtained the total UVA and UVB doses (DUV-A and DUV-B) as a function of the mean depth. The equations used were:

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

for the static treatments, and

$$D_{UV-X}(\bar{z}) = \int_t I_{UV-X}(t) \quad \exp(-K_d^{UV-X}\bar{z}(1 + \cos(wt)))dt$$

for the rotating treatments. The index 'X' stands for the different UV regions A or B, w is the angular velocity of the wheels ($\sim$0.026 s^{-1}). Note that because of the exponential attenuation of UVR with depth, the irradiance at the mean depth is lower than the mean irradiance experienced by the rotating treatments.

The calculations of predicted survival proceeded in two steps. First, we fitted a logistic model to the survival data using the UVA and UVB doses as independent variables:

$$d = \frac{\exp(k + a\text{UVA} + b\text{UVB})}{1 + \exp(k + a\text{UVA} + b\text{UVB})}$$

where d is the total mortality, k is the background mortality (i.e. the mortality when UVA and UVB are zero), and a and b are the coefficients for the UVA and UVB doses, respectively. We fitted the model using the Systat NONLIN procedure. In this first step, we used only the data from static incubations. In a second step, we used the parameterized model to calculate the predicted survival from the corresponding measured UVA and UVB doses.

Results and discussion

The survival of vertically moving *B.gracilipes* was accurately predicted using the information from static incubations (Figure 6A). The agreement between these results and the results obtained in a previous study (Zagarese *et al.*, 1998)
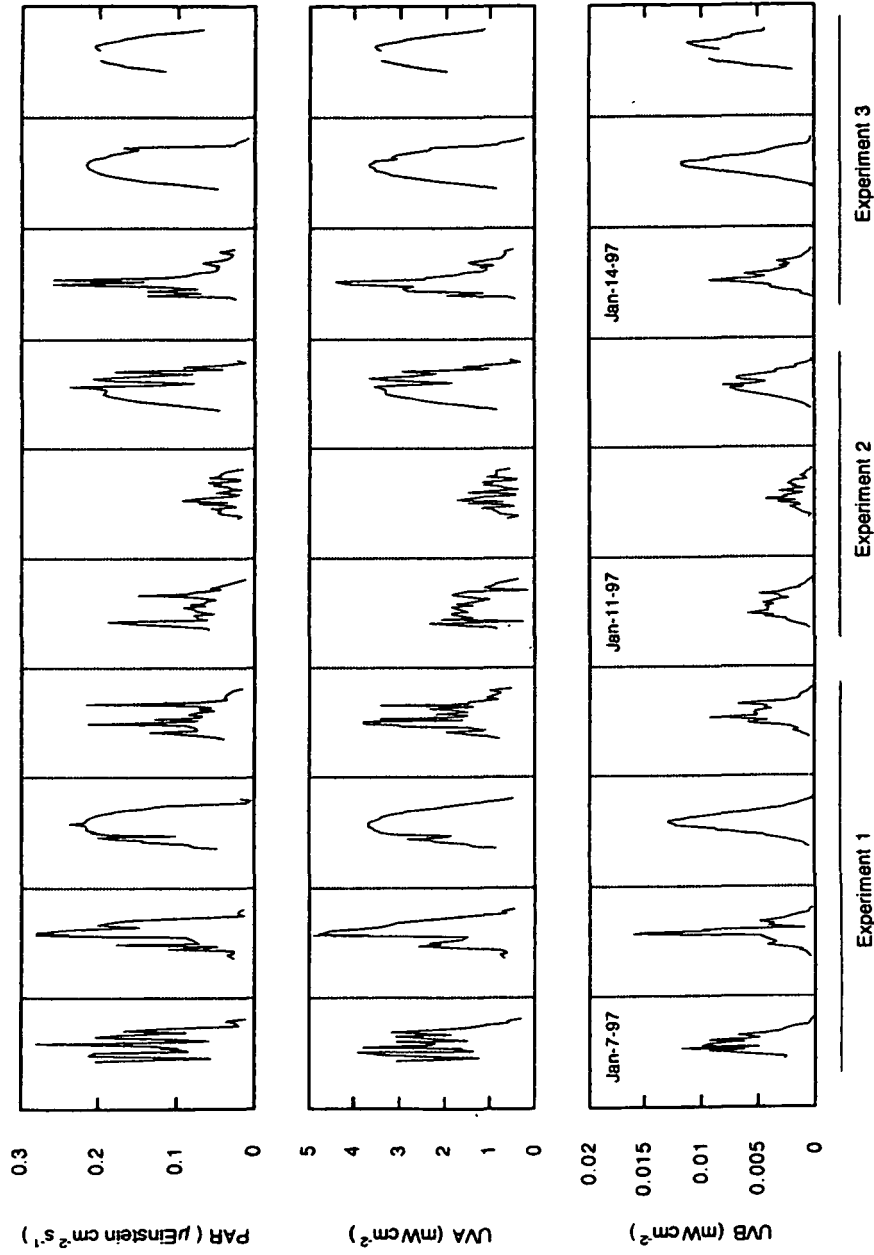
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Fig. 4. Irradiance conditions during the experiments in Lake Trébol.

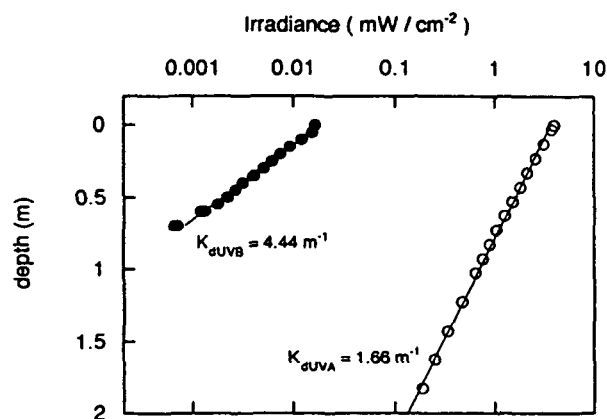Irradiance (mW / cm^{-2})

Fig. 5. Radiation attenuation profiles in Lake Trébol.

strengthens our confidence in the reproducibility of these experiments. In contrast, the experiments using *C.dubia* produced radically different results. Static incubations failed to predict the survival of vertically moving individuals to any significant extent. In addition, it should be noted that the results departed from reciprocity and were, in fact, opposite to what was expected. That is, at comparable doses, the survival of vertically moving individuals tended to be higher than that of static individuals, particularly at high doses, i.e. low survival (Figure 6B). The same pattern was observed in the three independent experiments with *C.dubia*. As the experiment with *B.gracilipes* and the first experiment with *C.dubia* were run side by side, we have no reason to suspect that the pattern observed for *C.dubia* could have been due to an artifact, such as a systematic error in calculating the doses.

Another important difference between the two species was the much higher within-treatment variance observed in *C.dubia*. Moreover, the high variance observed in the field experiments with *C.dubia* contrasts with the relatively low variance observed in laboratory experiments with the same species (Figure 7). We suggest that a substantial part of the within-treatment variance observed in *C.dubia* may be the result of individual differences in photorecovery, which seems to be stimulated by fluctuating radiation levels. This source of variation is less likely to be detected in studies with small organisms (i.e. bacteria, algae), in which the response variables usually represent the average performance of many individuals (e.g. carbon uptake). Nevertheless, large variation in DNA damage in individual cells has been demonstrated using flow cytometry techniques in a species of algae capable of photorecovery (Buma *et al.*, 1995, 1996).

It is interesting to note that the hypothesized increase in photorecovery as a consequence of fluctuating radiation levels with the consequent increase in stochasticity provides a potential mechanism for the contrasting responses between the two species. The results from these and previous experiments with *B.gracilipes* (Zagarese *et al.*, 1998) strongly suggest that UV damage is mainly a
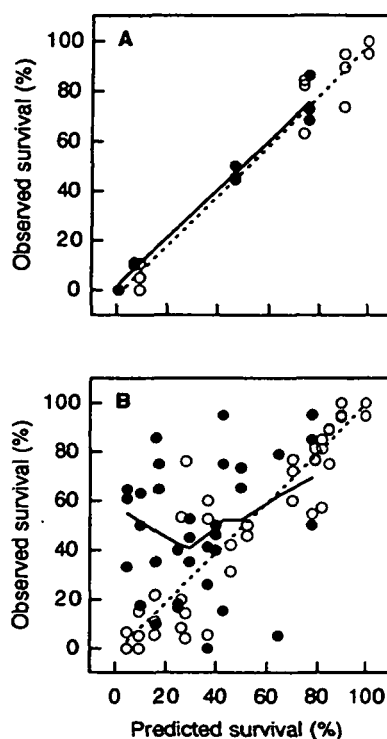
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Fig. 6. The relationship between observed and predicted survival for (**A**) *B.gracilipes* and (**B**) *C.dubia*. Predictions are based exclusively on information from static incubations assuming that reciprocity is satisfied. For *B.gracilipes*, the predictions based on static incubations accounted for 97% of the variance in observed survival for both static (open circles) and moving treatments (open circles). For *C.dubia*, the proportion of explained variance was 83% for static incubations and as little as 2% for moving treatments. The broken solid line represents a locally weighted linear regression showing that the observed survival of *C.dubia* in rotating treatments tends to be higher than predicted, particularly at low survival (i.e. at high UV doses).

function of dose with a rather minor stochastic component (Figure 6A). On the other hand, differences in photorecovery among individuals would result in greater data variability, which would be more evident when the damage is large and the environmental conditions for photorecovery are optimal. Therefore, if fluctuating radiation levels stimulate photorecovery, this should translate into three simultaneous effects: (i) an increase in the within-treatment variance; (ii) an overall increase in survival; and (iii) the previous two effects will be more apparent at higher doses, because the influence of photorecovery is greater when the amount of damage is large. Clearly, this could account for the differences between the two species, as well as between static and rotating treatments in *C.dubia* (Figures 6 and 7).

In order to provide an illustration of the previous mechanism, we generated survival data using Montecarlo computer simulations. The program starts by calculating the probability that an organism will die as a function of the UV dose
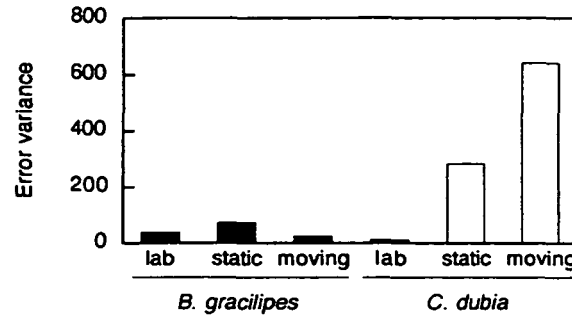
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Fig. 7. Within-treatment variance in different experiments. The error variance was calculated as the 'pure error' mean square (MSPE) (Neter and Wassermann, 1974), which is a measure of the average deviation of the observations from their corresponding treatment mean. Dark bars, experiment in which photorecovery was prevented (i.e. laboratory experiments with *C.dubia* without recovery radiation, or experiments with *B.gracilipes* which lacks photorecovery); open bars, experiments in which photorecovery could act (i.e. field experiments with *C.dubia*).
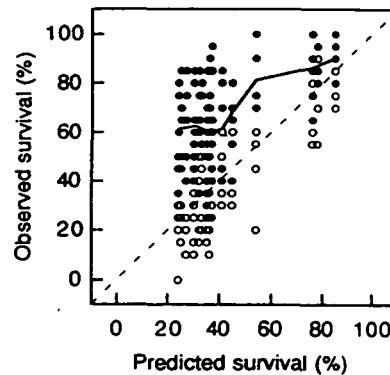
Fig. 8. Results from Montecarlo computer simulations. Each dot represents the percentage of surviving individuals in a 'tube' having 20 'individuals'. Symbols are as in Figure 6.

(non-random, damage component). In a second step, the probability of dying is reduced by a random amount, which is intended to simulate variations in photorecovery among individuals. The effect of photorecovery is assumed to be higher in rotating treatments. Finally, the program decides whether an individual dies or survives by comparing the probability of dying calculated in the previous step against a second random number. The raw survival data provided by this procedure were subsequently analyzed in the way already described for the experimental data. The results (Figure 8) are remarkably similar to the survival pattern observed in *C.dubia* (Figure 6B).

Conclusion

It is evident that more detailed knowledge on photorecovery kinetics is required. Nevertheless, regardless of the actual mechanism behind the observed pattern, the empirical evidence collected in these experiments shows conclusively that, for certain species, static incubations fail to predict the performance of vertically moving individuals, and may offer a distorted picture of the effects of UVR in nature.

Acknowledgements

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