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Motility of *Daphnia spinulata* as Affected by Solar Radiation Throughout an Annual Cycle in Mid-latitudes of Patagonia†

Rodrigo J. Gonçalves*, Elena S. Barbieri‡, Virginia E. Villafañe and E. Walter Helbling

Estación de Fotobiología Playa Unión (EFPU) & Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Playa Unión, Rawson, Chubut, Argentina

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ABSTRACT

During an annual cycle, *Daphnia spinulata* collected from a plankton community of Patagonia was exposed outdoors to assess the impact of recently received solar radiation on motility (*i.e.* swimming speed and gravitaxis). Individual values of these parameters were obtained by video recordings and image analysis at different time intervals during the day. Initial swimming speed varied throughout the year, and changes in speed during exposure were not significantly affected by any waveband used in our experimental design (*i.e.* PAB, 280–700 nm; PA, 320–700 nm; and P, 400–700 nm). Overall, most of the individuals swam downwards, regardless of the radiation treatment imposed to the samples. We found that multifactor interactions (*i.e.* not a single parameter explained more than 40% of the observed variability) explained most of our observations on motility parameters. These factors include not only solar radiation, but other physical (underwater radiation field and wind intensity) and biological parameters (food availability, presence of predators and congeners). Our findings indicate that the plankton dynamics in the study site is likely to be governed by a sum of factors which must be taken into account when considering solar radiation effects on aquatic ecosystems.

INTRODUCTION

Cladocerans of the genus *Daphnia* usually dominate the zooplankton community in freshwater ecosystems, and thus they are responsible for a significant portion of biomass' secondary production (1). In the water column, these plankton organisms are exposed to solar radiation, both photosynthetic active radiation (PAR; 400–700 nm) and ultraviolet radiation (UVR; 280–400 nm). During the last decades and after the discovery of the Antarctic ozone "hole" with the concomitant increase of ultraviolet radiation B (UV-B; 280–315 nm) (2), the effects of UVR in aquatic ecosystems and organisms have been the focus of intensive research (3,4). These studies have demonstrated that UVR can harm organisms, mainly through direct damage to the DNA molecule, proteins and membranes (5).

Previous studies indicated that UVR affects the performance of zooplankton organisms inhabiting the aquatic environment (6,7). Although we have learned much about the effects and potential impacts of UVR on zooplankton, however, relatively little is known about the sublethal effects of these short wavelengths on more subtle aspects such as motility. For example, it has been observed in laboratory studies that *Daphnia magna* swims away from light and changes its behavior when exposed to monochromatic UVR (especially in the ultraviolet radiation A (UV-A) range, with a maximum spectral sensitivity at 340 nm), while the opposite reaction is observed when individuals are exposed to visible light (8). Individual motility of zooplankton is involved in several activities that collectively are fundamental for the next cohort such as escape from predators, male–female encounter, feeding appendage movements and vertical migration (9–13). Motility can also be evaluated in terms of the tendency of individuals to swim towards or away from the water surface (*i.e.* vertical migration) producing a change in the radiation exposure of organisms. In this sense, previous studies showed surface UVR avoidance by *Daphnia cucullata*, *Daphnia rosea* and *Daphnia pulex* (both, pigmented and nonpigmented individuals), using natural and artificial radiation sources (14). Other studies of vertical migration have shown that *Daphnia pulicaria* has a downward migration when natural UVR is present, while individuals tend to remain closer to the surface in the UVR-shielded treatment, even at the risk of exposure to visual predators (15). Invertebrate predators such as *Chaoborus* can interact with UVR to make *Daphnia catawba* vertical migration behavior more complex (16). Furthermore, *D. catawba* individuals exposed to natural UVR show deeper distribution than they would probably have if no UVR was present (17). Thus, it is likely that changes in individual motility would become a potential source of variations in the zooplanktonic community.

The aim of this study is to assess the impact of recent exposure to solar radiation on motility (*i.e.* swimming speed and orientation) of the cladoceran *D. spinulata* Birabén, 1917 from a freshwater body of the Chubut Province, Argentina. The results obtained in this study correspond to the worst-case scenario for these organisms inhabiting mid-latitudes, as in the eastern part of Patagonia (*i.e.* away from the Andes mountains) most freshwater bodies are permanently exposed to solar radiation due to the absence of trees or tall buildings. This study complements the database on photobiology of

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‡Present address: Elena S. Barbieri, Ministerio de Educación de la Provincia del Chubut, Argentina.

*Corresponding author email: rodrigo@efpu.org.ar (Rodrigo J. Gonçalves) freshwater zooplankton organisms from the Patagonia area, where studies devoted to assess surface avoidance, survival, photoreactivation, production and presence and role of UV-absorbing compounds have been carried out (18–23).

MATERIALS AND METHODS

Study site/sampling. This study was carried out at the shallow, eutrophic, Cacique Chiquichano Lagoon located at eastern, central Patagonia, in the city of Trelew, Chubut, Argentina (43° 14′ S and 65° 18′ W). This freshwater lagoon is the largest (0.18 km^2) water body within the city, with a mean depth of ~2 m; it is located within the populated area so that it is a very accessible sampling site. The absence of trees or buildings in the surroundings not only lets full solar radiation reach the lagoon, but also allows exposure to winds all year round (24) bringing about mixing of the water column (25). In addition, the area is periodically under the influence of ozone-depleted air masses (*i.e.* during October–November) associated to the polar vortex (26). Some studies on photobiology of zooplankton (20) and phytoplankton (27) have been already carried out at the Cacique Chiquichano Lagoon.

Sampling and experimentation were performed throughout an annual cycle, from February 2005 to January 2006, with a frequency of once every 2–4 weeks. Zooplankton samples were collected using a net (200 μm mesh size) that was horizontally towed in the upper 20 cm of water column. The specimens were carried immediately to the laboratory at Estación de Fotobiología Playa Unión (EFPU, 30 min away from the sampling site) in 2 L polycarbonate bottles inside a thermal/light-isolated container. At the laboratory, zooplankton was kept overnight inside a culture chamber with controlled temperature at 18°C until the following morning, when motility experiments were performed (see below). It should be noted that in previous studies (and also in the data presented below), we found the maximum concentrations of *D. spinulata* around 18°C so this is why we set up our experimentation at that temperature all year around. We are aware that in this way we are not determining the potential impact of solar UVR at *in situ* temperature. Instead, we considered it more important to compare the responses to solar radiation of temperature-acclimated organisms at various times of the year. In doing so, we are ruling out the influence of temperature in metabolic processes and thus any potential differential recovery throughout the year due to this factor. Thus, our experiments focused on the sublethal effects of UVR on *D. spinulata* when other variables such as the previous light history, quantity and quality of food and presence of other zooplankton species were also changing throughout the year.

Surface samples were additionally collected with a clean bucket (1 *N* HCl) for determination of other parameters such as phytoplankton abundance, chromophoric-dissolved organic matter (CDOM) and chlorophyll a (chl a) concentration (see below).

Zooplankton exposure and filming. Before experimentation, moribund and dead individuals were removed with a pipette from the original sample; we defined as moribund those individuals that did not perform any swimming or locomotive movements when disturbed, even though they might show movements on the filtering appendages. Experiments were carried out with the entire zooplankton community collected, that consisted typically of specimens of the cladoceran *D. spinulata* (both males and females) and the cyclopoid copepod *Metacyclops mendocinus* (Wierzejski, 1892); however, only motility data obtained with *D. spinulata* are presented in this study and *M. mendocinus* data will be included in a future work.

Zooplankton specimens were gently divided and randomly distributed into eight UV-transparent polycarbonate chambers (Plexyglas® XT-Röhm GmbH & Co. KG, Darmstadt, Germany) filled with filtered (100 μm mesh) water from the lagoon. The chambers are 23 × 16 × 3 cm (length × width × depth) and were built especially for filming free-moving individuals while keeping a relative small focal depth (3 cm). In our exposure setup, the samples were contained in a 3 cm depth enclosure. Three radiation treatments were implemented (duplicates for each treatment) by covering the chamber with different filters: (1) P treatment: samples receiving only PAR (400–700 nm)—chambers covered with Ultraphan 395 (UV Opak, Digefra; 50% transmission at 395 nm), (2) PA treatment: samples receiving PAR + UV-A (315–400 nm)—chambers covered with Ultraphan 320 (UV Opak, Digefra; 50% transmission at 320 nm) and (3) PAB treatment: uncovered chambers, receiving PAR + UV-A + UV-B (full solar radiation, 280–700 nm). Additionally, two chambers were wrapped with aluminum foil and used as "dark" controls. All chambers were placed in a bath with running water for temperature control (18°C ± 2°C) and exposed horizontally to solar radiation (clear sky, no shading) for 8 h (with incubations starting at 9 h in the morning). At time zero and then, every 2 h (*i.e.* 2, 4, 6 and 8 h of exposure) chambers were taken one by one and gently rotated 90° and placed vertically in a dark housing (to avoid phototactic movements) with only a white diffuse background to film swimming patterns of zooplankton (therefore, UVR exposure and filming were not simultaneous). With the rotation of the chambers, we were able to frame the whole surface that was exposed to solar radiation (*i.e.* 23 × 16 cm) and *D. spinulata* could swim up or down a larger distance (16 cm depth during filming instead of the 3 cm depth during exposure) and thus we could follow the specimens in the whole chamber. Manipulation of chambers was careful and gentle enough to avoid mechanical disturbance of swimming patterns of organisms while being filmed. As soon as the chamber reached the "filming position" (*i.e.* in less than 60 s from the time that samples were taken from the exposure water bath) motility of zooplankton was recorded during ~120 s with a video camcorder (JVC–GR-AX4) and then returned to the exposure rack (the whole procedure took less than 5 min); each experiment was recorded and stored into VHS-C tapes. A 1 cm length reference scale was included in each chamber to be seen at all times during filming. Initially, we separated and analyzed the video at 30 s intervals, but there were no differences in the measured parameters among each interval, so we used the whole recording time in all our analyses.

Image analyses. Video recordings were analyzed using image analysis software (Wintrack2000, Real Time Computers, Inc., Germany) (28). Besides speed and orientation of movement, the software assigns to each individual a "form factor" (a parameter to distinguish round from long objects) which allowed us to sort out data from cladocerans (rounded shape) and copepods (long shape). Preliminary tests were performed using separate *M. mendocinus* and *D. spinulata*, and from the data a form factor characteristic to each species was determined. The form factor interval for *M. mendocinus* ranged from 1.23 to 1.77 (mean ± 3 SD), whereas that for *D. spinulata* ranged between 2.21 and 2.48 (mean ± 3 SD). We assigned a limit form factor of 1.94 with all data below it corresponding to *M. mendocinus*, while those above it corresponded to *D. spinulata*. The limit form factor chosen allowed us to completely separate both species, and this was also further confirmed (*i.e.* all form factors were within the assigned limit) at times when one species completely dominated the zooplankton and the other was not present.

The angle of movement (*i.e.* direction, in degrees) associated with each velocity is 0° for individuals swimming towards the surface, and 180° for individuals swimming towards the bottom. Thus, all vertical movements were divided into upward (angles $>270°$ or $<90°$) and downward swimming (angles between 90° and 270°). In this way, we defined a "swimming direction" parameter that considers the number of individuals moving upward or downward. This swimming direction parameter for each sample was calculated as: ($[N/Nt] \times 100$), where N denotes the number of individuals swimming downward or upward and Nt is the total number of individuals registered in the film. N was considered negative for downward movements and positive for upward swimming. The software also computed some values ($<5\%$ of individuals) as pure horizontal movements (*i.e.* 90° or 180° swimming direction) that were not considered in our calculations. As each individual of the same *Daphnia* population can have different migration trends (29), a resultant swimming direction (*i.e.* the sum of negative and positive values obtained) was calculated to obtain a measurement of the net orientation of the entire sample group. For example, if 40% of the individuals were swimming upwards and 55% were swimming downward, the resultant swimming direction would be −15. The resultant swimming direction did not vary significantly throughout any experiment ($P > 0.05$, repeated measures ANOVA) so we used an average value for each treatment, calculated from the beginning of the experiment until 1:00 P.M. (maximum radiation) as representative of the whole experimentation day.

Zooplankton abundance. Samples for determination of abundance of zooplankton in the natural community were fixed either with formalin (20%) or ethanol (96%) and counted under a dissecting microscope (magnification = 40×).

Photosynthetic pigments. Chl a concentration of prefiltered water (100 μm mesh, to exclude crustacean zooplankton) was obtained by filtering between 10–250 mL of sample onto a Whatman GF/F glass fiber filter (25 mm) and extracting photosynthetic pigments in absolute methanol (30). The fluorescence of the methanolic extract was measured using a Turner Designs fluorometer (model TD700) before and after acidification; chl a concentration was then calculated from these readings (31). The fluorometer was calibrated using pure chl a from *Anacystis nidulans* (Sigma No. C 6144). Chl a in the nanoplankton fraction (<20 μm) was obtained by prefiltering the sample with a 20 μm mesh net and processed as above. The relative nanoplankton content (%) was calculated as ($[\text{chl a}_{<20\,\mu m}]/[\text{chl a}_{\text{Total}}]) \times 100$.

Chromophoric-dissolved organic matter. After filtering 10 mL of water onto a Whatman GF/F glass fiber filter (25 mm), the optical density (OD) at 320 nm of the filtrate was determined with a spectrophotometer (Hewlett Packard model HP 8453E) using a 5 cm path length cuvette. The absorption coefficient at 320 nm (a_{320}) used as estimation of the CDOM was then calculated according to Osburn and Morris (32) as:

$$a_{320}\ (\text{m}^{-1}) = 2.303 \times \text{OD}_{320}\,\text{L}^{-1},$$

where OD_{320} is the OD at 320 nm and L is the length of light path inside the cuvette (0.05 m).

Solar radiation and other atmospheric parameters. Solar radiation was continuously monitored (*i.e.* one datum per minute) with a broad band filter radiometer (ELDONET, Real Time Computers, Inc.) that measures UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm). During the study period, wind speed and direction were monitored every 10 min with a meteorological station (Oregon Scientific model WMR-918). Wind speed used in the predictive model (see below) represents the average of the 3 days previous to sampling at the Cacique Chiquichano Lagoon (wind data were not available on day 13 due to technical problems). Ozone values were obtained from the NASA database (http://jwocky.gsfc.nasa.gov).

Estimation of the underwater radiation field. Because of the impossibility of carrying out *in situ* measurements of underwater radiation (due to the lack of appropriate equipment for measurements at this shallow site), we estimated the diffuse downwelling attenuation coefficient for PAR (K_{PAR}) using previously published models (33,34) as:

$$K_{\text{PAR}} = (0.22 + 0.008 \times \text{chl a} + 0.054 \times \text{chl a}^{0.66}) + (0.1948 \times a_{320} - 0.9203),$$

where the first term of the equation represents the relationship of K_{PAR} with chl a and the second that with CDOM. The first term is in the form $K_0 + 0.008\ (\text{chl}) + 0.054\ (\text{chl})^{2/3}$ which has been used to predict K_{PAR} in several eutrophic and mesotrophic lakes (33). As background attenuation (K_0) was unknown, we adopted the value of 0.22 for mesotrophic Lake Ontario as used by Thomann *et al.* (cited in Branco and Kremer, 33). For the second term, we used published data from Morris *et al.* (34) of a group of lakes with high-dissolved organic carbon, but relatively low chl a, and similar range of absorption (a_{320}) as observed for Cacique Chiquichano Lagoon (*i.e.* Alaskan lakes) to regress the measured K_{PAR} on a_{320} ($R^2 = 0.74$, $n = 13$). While the accuracy of this model is unknown (particularly because of uncertainty in K_0), we believe that it should provide a good qualitative description of PAR attenuation in the lagoon.

Taking into account the shallowness of the Cacique Chiquichano Lagoon and that wind is generally present throughout the year (35), we assumed that it was well mixed to the bottom. Under these conditions, we calculated the mean underwater PAR irradiance (Im) according to Helbling *et al.* (36) as

$$\text{Im} = I_0\left[1 - \exp(-K_{\text{PAR}} \times z)\right]/K_{\text{PAR}} \times z,$$

where I_0 is the maximum PAR irradiance at the surface at local noon, K_{PAR} the attenuation coefficient and z the depth of the lagoon (0.8 m) at the sampling site.

Statistics. All experiments were performed in duplicates and routinely 100–200 individuals per chamber were tracked to obtain a mean and a standard deviation for each radiation treatment. On several occasions, when the concentration of *D. spinulata* was low, fewer individuals were tracked, but at least 10 were followed. Each chamber was considered an experimental unit (statistical sample). Two types of analysis of variance (ANOVA) tests were used for different time-scales: (1) Within each experimentation day, a repeated measures ANOVA was used, whereas (2) A two-way ANOVA test was used to test differences between radiation treatments and dates during the year. A significance level of 0.05 was used in all analyses. When comparing results from the exposure before and after noon, we used paired samples *t*-test. When regression analysis was performed, the best fit and the 95% confidence limits were reported together with R^2 and P values. For the regressions, we did not use any of the backward or forward methods but rather, all the independent variables of the model were entered in a single step.

RESULTS

Incident solar radiation and ozone concentration over the Cacique Chiquichano Lagoon during the year 2005 are presented in Fig. 1. Surface radiation patterns in terms of daily doses (Fig. 1a, b and c) are in agreement with the typical distribution at mid-latitudes (*i.e.* with high values during summer and low during winter) due to the gradual change of the earth's proximity and orientation relative to the Sun. PAR irradiance at local noon and daily doses (Fig. 1a) displayed a similar pattern throughout the year, with maximal values of ~450 W m^{-2} and ~12 000 kJ m^{-2} on Julian days 328 and 4, respectively. Daily doses of UV-A and UV-B (Fig. 1b and c) followed the same trend as that of PAR, with maximum values during summer and minimum in winter. Maximal daily doses were measured during summer, with values of 1830 and 50 kJ m^{-2} for UV-A and UV-B, respectively. Minimum values were found during mid-winter, with doses of 21 and 0.2 kJ m^{-2} for UV-A and UV-B, respectively (Fig. 1b and c). The day-to-day variability observed in PAR and UVR patterns was mostly due to changes in cloud cover. Ozone concentration over the lagoon (Fig. 1c) varied throughout the year, with low values during summer and autumn and relatively high ones during winter and spring. The maximum concentration was observed during winter, with 405 Dobson Units (DU; on Julian day 217) as the highest value, whereas the lowest value (231 DU) occurred on Julian day 120. During the study period, average wind speed varied between 0 and 5.8 m s^{-1}, with a maximum value of 15 m s^{-1} (data not shown); the strongest winds occurred normally during autumn and spring.

Some biological and physical characteristics of the water body are shown in Fig. 2. Chl a concentration varied significantly throughout the year (Fig. 2a) with high values in January (*i.e.* summer, 663.7 μg L^{-1}), April (*i.e.* autumn, 778.4 μg L^{-1}) and August (*i.e.* winter, 578 μg L^{-1}). Relatively low chl a concentrations were determined in between these months, with values as low as 31.7 μg L^{-1} in June (*i.e.* winter) and 4.2 μg L^{-1} in December (*i.e.* spring). The absorption coefficient (a_{320}), used as an estimator of CDOM (Fig. 2a) had relatively less variability than chl a concentration, with a trend of increasing values as the year progressed. The minimum a_{320} value (*i.e.* 3.4 m^{-1}) was determined during February (*i.e.* summer), whereas the maximum (*i.e.* 23.6 m^{-1}) occurred during September (*i.e.* late winter). The estimated attenuation coefficient for downwelling PAR (K_{PAR}) varied between 2.2

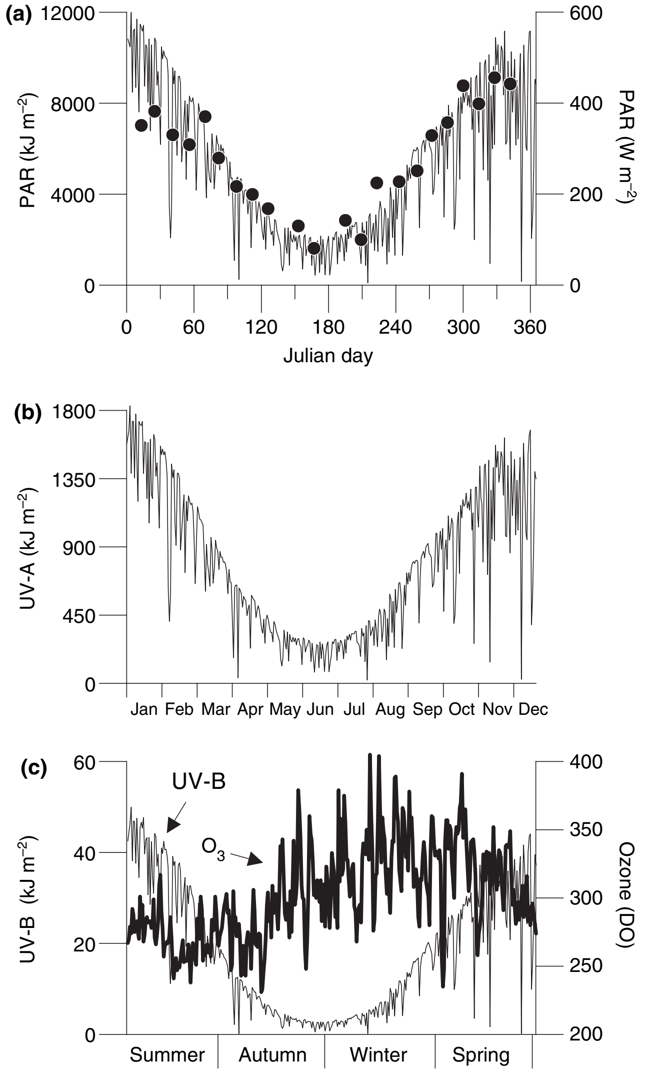

Figure 1. Selected atmospheric parameters as a function of time at the Cacique Chiquichano Lagoon. (a) Daily dose (solid line, in kJ m^{-2}) and irradiance (circles, in W m^{-2}) of PAR (400–700 nm) at surface. (b) Daily doses of UV-A (320–400 nm) and (c) Daily doses UV-B (280–320 nm) (thin line, in kJ m^{-2}) at the surface, and ozone concentration over the site (thick line, in DU). Ozone data was obtained from http://jwocky.gsfc.nasa.gov. For clarification purposes, Julian day, months and seasons are shown in a, b and c, respectively.

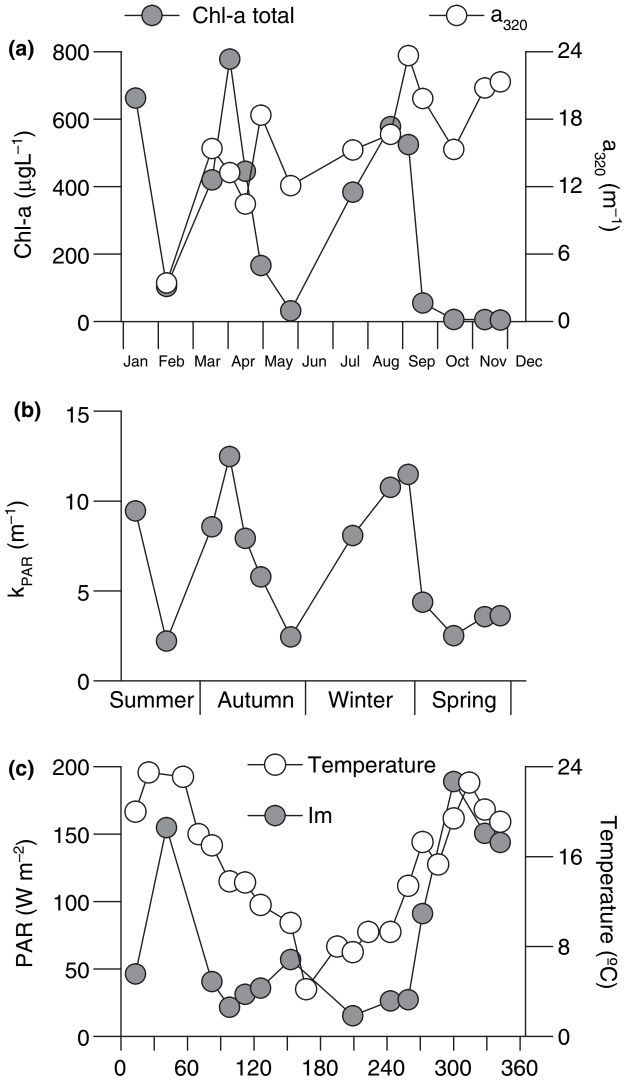

Figure 2. Biological and physical parameters at the Cacique Chiquichano Lagoon throughout the annual cycle. (a) Phytoplankton abundance, estimated by chlorophyll a (chl a) concentration (dark circles, in μg L^{-1}) and chromophoric-dissolved organic matter, CDOM (estimated by the absorbance at 320 nm) (white circles, in m^{-1}), (b) Estimated attenuation coefficient of downwelling PAR (in m^{-1}) and (c) Estimated mean underwater PAR irradiance, Im (dark circles, in W m^{-2}) and water temperature (white circles, in °C).

and 12.5 m^{-1} (Fig. 2b). According to the model used in this study, low values of chl a and a_{320} resulted in low values for K_{PAR} (*e.g.* on Julian days 41, 153 and 300); the highest K_{PAR} were estimated to occur during January, April and September (*i.e.* on summer, autumn and late winter, respectively) and they were associated to high chl a values. The estimated mean underwater irradiance (Im, Fig. 2c) remained relatively low during autumn and winter (*i.e.* Julian days 82–259) with values < 57 W m^{-2}. During summer and spring, however, Im increased, reaching values of 155 and 189 W m^{-2} on Julian days 41 and 300, respectively. Finally, water temperature at the Cacique Chiquichano Lagoon (Fig. 2c) had a wide range of values throughout the year, ranging from > 23.5°C during the summer to 4.2°C in winter, even with the formation of ice during some mornings.

The relative abundance of the two representative zooplankton species (in terms of quantity and biomass) is presented in Fig. 3. Herbivorous cladocerans (almost exclusively represented by *D. spinulata*) and the omnivorous copepod *M. mendocinus* were alternatively dominant throughout the year (Fig. 3). Copepod abundance was relatively high (> 100 ind L^{-1}) during January, April and July (summer, autumn and winter, respectively), when they accounted for more than 60% of the zooplankton abundance; their concentration, however, was low during May–June (autumn) and September–November (spring) (< 30 ind L^{-1}). The maximum concentration of *M. mendocinus* was determined during April (*i.e.* > 210 ind L^{-1}). On the other hand, cladocerans clearly dominated during February (summer), May–June (autumn) and September–November (spring) when they accounted for more than 75% of

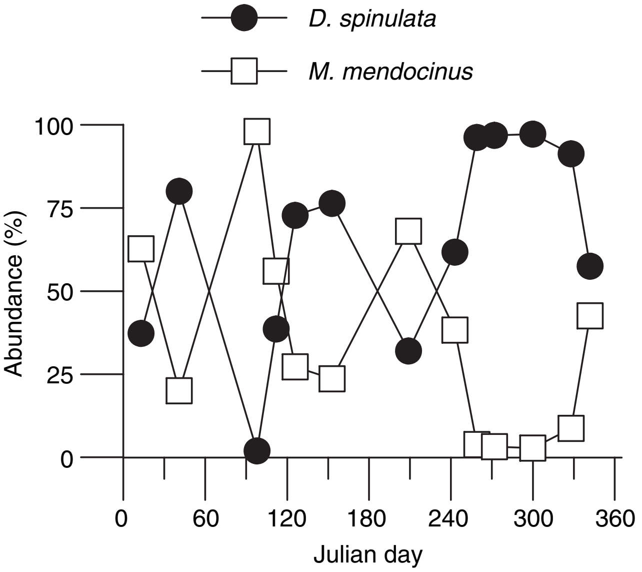

Figure 3. Relative abundances (%) of the cladoceran *Daphnia spinulata* and the copepod *Metacyclops mendocinus* throughout the study period.

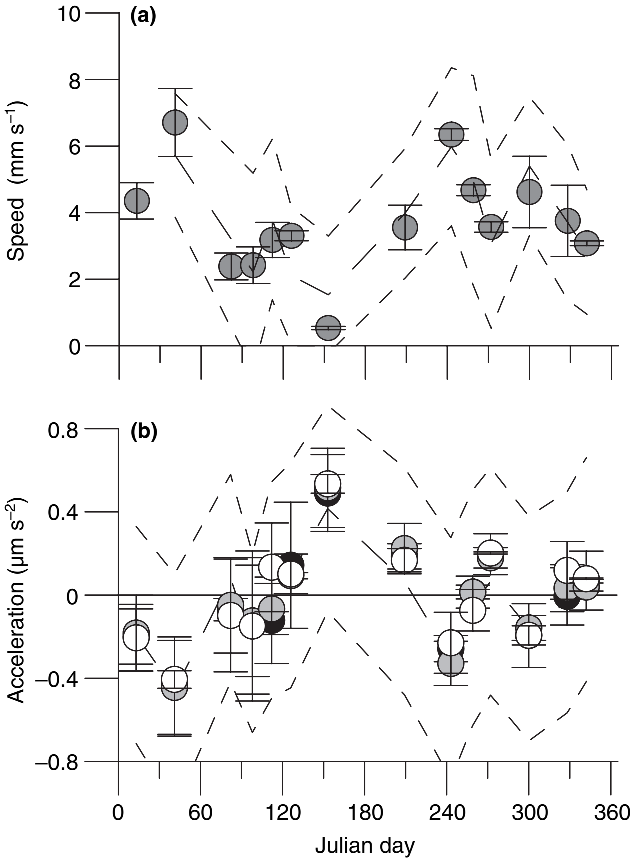

Figure 4. (a) Initial swimming speed (circles, in mm s^{-1}) of *Daphnia spinulata* measured during the study period. Error bars indicate 1 SD. The adjusted model and 1 SD are shown in broken lines; (b) Swimming speed changes (*i.e.* acceleration) of *D. spinulata* (circles, in µm s^{-1}) calculated from experimental data (*i.e.* from time zero to 1:00 P.M.). Black circles are samples exposed to full sunlight (PAB treatment, 280–700 nm); Gray circles denote samples that received radiation in the 320–700 nm interval (PA treatment), and white circles are samples exposed only to visible radiation (P treatment, 400–700 nm). Broken lines show the predicted acceleration according to the adjusted model and 1 SD.

the abundance and reached maximum concentrations of 377 ind L^{-1} during September (concentration data not shown).

The motility patterns of *D. spinulata* are shown in Figs. 4 and 5. The initial swimming speed (*i.e.* before exposure) varied considerably throughout the year and ranged between 0.5 ± 0.05 mm s^{-1} (*i.e.* in samples collected during June) to 6.7 ± 1.01 mm s^{-1} (*i.e.* in samples collected during February) (Fig. 4a). We modeled the initial swimming speed of *D. spinulata* throughout the year based on its abundance, chl a concentration and previous wind speed. The reason for this calculation was to roughly estimate wind-induced water movements at the Chiquichano Lagoon that could influence zooplankton motility. The multiple linear regression obtained ($R^2 = 0.83$; $P = 0.002$) to estimate the initial swimming speed was:

$$\mathrm{Sm} = 3.199 + 0.003 \times (\mathrm{Ch}) - 0.574 \times (W) + 0.007 \times (D)$$

where Sm is the modeled initial swimming speed (mm s^{-1}), Ch is total chl a concentration (µg L^{-1}), W is the mean wind speed (m s^{-1}) previous to experimentation and D is the abundance of *D. spinulata* (ind L^{-1}).

During each day of experimentation, there were significant changes in the swimming speed as compared with the initial values (repeated measures ANOVA, $P < 0.05$ in all cases). These changes in swimming speed (*i.e.* acceleration) of *D. spinulata* exposed to different radiation treatments are shown in Fig. 4b. Acceleration patterns in *D. spinulata* varied significantly throughout the year ($F_{13,42} = 11.53$, $P < 0.01$), with positive values (*i.e.* increasing swimming speed) during late autumn/early winter, and negative ones (*i.e.* decreasing swimming speed) throughout the rest of the year. No significant effects ($F_{2,42} = 0.56$, $P = 0.57$) of the different wavebands were determined throughout the year in the acceleration of *D. spinulata*, with samples exposed to visible radiation having similar values than those receiving additional UV-A and UV-B. We estimated acceleration using a multiple linear regression, with the initial swimming speed and the relative nanoplankton content as factors, using the following equation ($R^2 = 0.89$; $P < 0.001$):

$$A = -0.623 + 0.11 \times (N) - 0.115 \times (S_0)$$

where A is the predicted acceleration (µm s^{-2}), N is the relative abundance of nanoplankton (%) and S_0 is the measured initial swimming speed (*i.e.* before exposure to solar radiation, in mm s^{-1}).

In any given experiment, some *D. spinulata* individuals swam upwards, while others moved downwards (Fig. 5). Averaged values from morning exposure (*i.e.* until *ca* 1:00 P.M.) were not significantly different ($T_{14} = 1.76$, $P > 0.9$) from that in the afternoon, thus morning values are presented in this study as representative for the whole experiment. There were no significant differences in the proportion of individuals moving upwards or downwards under full solar radiation (PAB) as compared with that under PA or P treatments ($P = 0.17$), so we pooled all the dataset for further calculations. The proportion of individuals

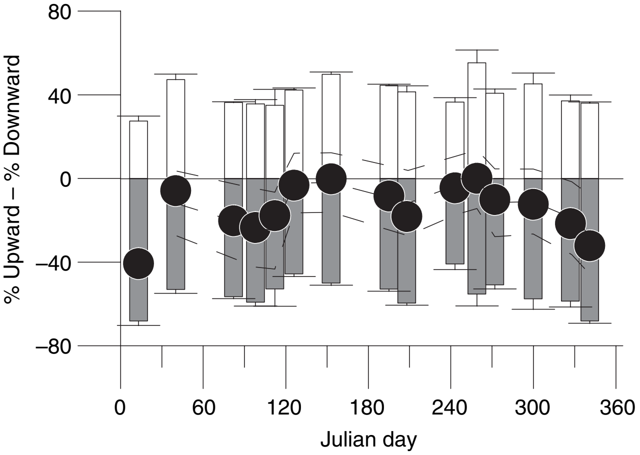

Figure 5. Overall swimming direction (black circles) obtained as the sum of the percentage of individuals swimming upwards (white bars, positive values) and the percentage of individuals moving downwards (dark bars, negative values). Error bars indicate 1 SD.

swimming upwards or downwards varied throughout the study period (bars on Fig. 5) but for most of the year there was an overall predominance of downward swimming (*i.e.* maximum of 68%) as compared with upwards (*i.e.* maximum of 55%). The resultant swimming direction (circles in Fig. 5) was downwards, with an extreme value of −41. Nevertheless, during late autumn–winter, the resultant was significantly different ($P = 0.002$), with a lower proportion of individuals swimming downwards than that during spring–summer, when the net direction was mostly away from the surface. The resultant swimming direction was best explained by a model based on diverse parameters: The fitted coefficients for the multiple regression indicated that increases in the abundance of the copepod *M. mendocinus*, wind speed and mean underwater irradiance resulted in a greater downward (*i.e.* negative) movement ($R^2 = 0.85$; $P = 0.001$), as follows:

$$G = 29.519 - 0.181 \times \mathrm{Im} - 0.145 \times \mathrm{Cp} - 5.979 \times W$$

where G is the resultant direction of individuals swimming upwards or downwards, Im is the mean underwater irradiance (W m^{-2}), Cp is the abundance of *M. mendocinus* (ind L^{-1}) and W is the wind speed (m s^{-1}) previous to experimentation.

DISCUSSION

The Patagonia region is periodically under the influence of low ozone air masses which have been detected in Argentina as far as 38°S of latitude (26). Therefore, the effects of UVR, especially of UV-B on aquatic ecosystems have received increasing attention, and several studies have been already conducted in this area (37). Motility of zooplankton is known to be affected by several environmental factors such as temperature (38,39), radiation (8,14–16,40), viscosity (41) and oxygen concentration (42). Attempts have been made to relate single or few factors (*e.g.* light, food, temperature and predators) to swimming behavior, and a wide range of responses have been found (43,44). Our data indicate that the patterns of swimming, acceleration and gravitaxis of *D. spinulata* throughout the year cannot be satisfactorily explained by a single-factor relationship. This is in agreement with previous studies (45) in which the swimming speed of *D. pulicaria* varied throughout the year, but it was not related only to food concentration. In our study, however, most of the variability of the initial swimming speed of *D. spinulata* was related to chl a concentration, together with the previous wind and *D. spinulata* abundance (Fig. 4a). This might reflect the dependence of swimming speed on acclimation mechanisms (*i.e.* previous wind) as well as on behavioral responses (*i.e.* food concentration and presence of congeners).

Changes in swimming speed

One of the objectives of this study was to determine if *D. spinulata* differentially changed its swimming speed (*i.e.* acceleration) when recently exposed to solar radiation. Contrary to what we expected, no UVR effects on this parameter were found, suggesting that *D. spinulata* was able to cope with this waveband and counteract any potential UVR impact. Previous laboratory studies with a related species (*Daphnia menucoensis*) collected from our study site (20) showed a highly efficient DNA-repairing mechanism that was light dependent (photoenzymatic repair; PER). We cannot discard, however, other possibilities such as that the *Daphnia* species inhabiting the Chiquichano Lagoon lack sensory response to UVR or the response was so small that it was not detected by our experimental setup. Another potential source of variability in motility parameters could be the difference between the *in situ* and the experimental temperature. In fact, we used constant experimental temperature throughout the year to compare the impact of other variables among seasons. We are aware that in this way, we are precluding natural environmental changes, as *D. spinulata* is normally exposed to differential water temperature throughout the year as well as during the day (*i.e.* due to the relatively large day-to-night temperature differences in the region). The relative abundance of *D. spinulata* throughout the year (Fig. 3a) is such that this species dominated during periods (February and September–November) in which water temperature was close to the experimental temperature (18°C) so that our data can effectively be related to *in situ* responses. During autumn, however, a small peak of dominance of *D. spinulata* was observed and during this period the experimental temperature clearly deviated from that measured *in situ*. Assuming that there should be more motility responses to UVR if the organisms are sensitive to damage, a possible reason for the lack of UVR effects could be the constant temperature used in our experiments, so that *D. spinulata* might have had a higher DNA repair rate than it would at *in situ* temperatures. In fact, studies conducted with phytoplankton from the Cacique Chiquichano lagoon (46) indicated that they were more sensitive to UVR during winter. Differences between *in situ* and experimental temperature could be implied in the absence of UVR effects, as enzyme systems that repair UVR-induced DNA damage are temperature dependent. Studies carried out with freshwater zooplankton (47) showed a higher tolerance of *D. catawba* (which depends primarily on PER for their UVR tolerance) to UVR at elevated temperatures. Other authors, working also with *Daphnia* species from temperate lakes found greater net DNA damage at lower temperatures, providing further molecular evidence for the temperature dependence of

UVR-induced DNA damage (48). The temperatures used in those studies (5–25°C) are well within the normal *in situ* temperature measured at the Chiquichano Lagoon (Fig. 2c). Within this context, one could speculate that experimentation conducted at *in situ* temperature would yield to different results during winter but one has to be aware of the low irradiance levels (Fig. 2c) at that time of the year.

The relationship found between initial swimming speed and chl a probably reflects the fact that low food concentration is related to a higher *D. spinulata* motility in the lagoon. In fact, chl a negatively correlated to *D. spinulata* abundance (R^2 = 0.48, P < 0.009) but with a short lag (Figs. 2a and 3), suggesting that cladocerans put a strong grazing pressure when they are numerically important and dominate the population, as seen in other studies (49,50). The "clear-water phase" induced by *Daphnia* grazing increases in turn the levels of underwater UVR (Figs. 2b and 3). On the other hand, the negative relationship with wind (*i.e.* slow swimming speed when wind previous to experimentation was strong) could be associated to an energy-saving strategy: High motility is not necessary if the probability of finding food is high (*i.e.* in a highly turbulent water column due to enhanced encounter rates) (51). Finally, an increase in swimming speed with increasing *D. spinulata* abundance might reflect density-dependent effects: In fact, other authors have found that clearance rates of *D. pulex* are reduced in a crowded media, this being triggered by infochemicals released from crowded congeners and conspecifics (52). Thus, it can be hypothesized that a high concentration of individuals also stimulates *D. spinulata* to increase its swimming speed to be able to compete for food but probably using less energy in feeding movements.

Even though we did not find significant differences among the UVR treatments imposed to the samples, UVR cannot be excluded as part of an environmental signal, especially when considering vertical migration. The downward swimming direction of *D. spinulata* found throughout the year is a resultant, but it is interesting to note that the population could be composed of more than one clone, with more than one vertical movement *tendency* (in our case, almost every possible movement would fall into the "upward" or "downward" category, Fig. 5) as seen in another study (29) in which a single *Daphnia* population was divided into clones with different migration patterns. Thus, it is rather expected that the *D. spinulata* population might not have a clear response as a whole. Previous studies (53) found that UVR influences the vertical distribution and habitat partitioning of some zooplankton groups in lakes with a high UVR penetration in the water column ("high-UV lakes"), while other factors (*i.e.* predation and food limitation) may be more important in "low-UV lakes." In this sense the Cacique Chiquichano Lagoon could be considered as "low-UV" due to the high attenuation coefficient of PAR (Fig. 2b) and concomitantly, with low underwater UVR irradiance.

Acceleration patterns

In the present study, other ecological factors such as the relative abundance of nanoplankton seem to play an important role in the observed acceleration patterns (Fig. 4b). Similarly to the effect of chl a concentration on initial swimming speed, the high proportion of nanoplankton in the water column seems to induce the acceleration of *D. spinulata* individuals, in agreement with studies that showed that food concentration has species-specific responses in several *Daphnia* species (43). *D. spinulata* might behave differently under different food composition (54,55) and grazing on small cells may have a lower net energetic value for *D. spinulata;* thus the organism would need to increase its motility to acquire greater amounts. In addition, juveniles and adult individuals of *D. spinulata* needed to cope with the omnivorous *M. mendocinus* that may predate on them, similarly as occurring in other environments (56,57). If this was the case, avoidance behavior of *D. spinulata* against the presence of copepods might be expected. Furthermore, our results show that the swimming direction of *D. spinulata* was negatively related to the concentration of *M. mendocinus*, and that individuals tend to swim to deeper and darker waters. However, other variables also explained part of the variability in gravitaxis such as the mean PAR irradiance in the water column (*i.e.* the higher mean irradiance associated to a greater number of *D. spinulata* swimming downward, Figs. 2c and 5). Each of the variables used in the models were obtained independently and were related to the previous history (initial swimming speed), underwater radiation (Im) and biological parameters (*M. mendocinus* as predator/competitor).

Orientation of *D. spinulata*

Several studies (8,14–16,53) found responses of *Daphnia* in terms of orientation to UVR wavelengths. However, in the literature, it is not always clearly stated how fast *Daphnia* responds immediately after exposure to solar UVR. For example, responses were obtained after several hours (14,16) or after 10–15 min (15,53). In our experiments, the manipulation of the chambers in which *D. spinulata* was exposed to solar radiation was very quick (each chamber was out of the exposure rack in < 5 min, and the filming started at most 60 s after taken from exposure). One could argue that no UVR effects on motility were found because filming and exposure were not simultaneous. However, it is unknown how fast *Daphnia* responds to a change from strong to dim (or no) illumination, although one study (8) shows rapid (20–60 s) responses to changes in the wavelength of monochromatic light. Therefore, the lack of difference between radiation treatments could mean that there was either no effect of UVR exposure on direction of movement or that directional movement did occur when the chambers were in the light but this movement dissipated very rapidly when the organisms were transferred to the dark. Another alternative is that the effects can be seen in periods > 60 s, but we did not find differences between the initial and the last 30 s intervals of each 120 s filming session. Finally, we could think that the effects last more than the time span for filming the individuals and that recordings are representative of the average motility parameters for *D. spinulata* at that time. To be on the "safe side," we stated that there was no significant effect of the recently received UVR.

It has been shown that *D. magna* had both UVR- and PAR-induced phototactic responses (negative and positive, respectively) (8). In their natural environment, however, pelagic zooplankton is subjected not only to different wavelengths (damaging and repairing) at the same time and in varied proportions, but also to changes of variables such as predators and food. A set of environmental factors appears to be more important than the recent UVR when considering the swimming behavior of *D. spinulata*. This seems to be rather expected, as *D. spinulata* at the Cacique Chiquichano Lagoon is exposed to a highly variable environment that undergoes important changes in physical, chemical and biological variables throughout the year. Particularly, our data indicates that wind is an important factor in controlling the system that conditions swimming behavior of this species. Wind speed is possibly a stress factor in this region, although it is seldom taken into account when studying responses of zooplankton. Overall, this study suggests that a combination of parameters explains most of the observed variability in the swimming behavior of *D. spinulata* and that the impact of solar UVR is negligible at the sublethal effect level. However, direct effects of solar UVR on phytoplankton might be important in the behavior of *D. spinulata* through the selection of different size classes/species that constitute the food basis for this cladoceran.

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