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Ultraviolet radiation dose calculation for algal suspensions using UVA and UVB extinction coefficients

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ABSTRACT

Although the biological importance of ultraviolet light (UVR) attenuation has been recognised in marine and freshwater environments, it is not generally considered in in vitro ecotoxicological studies using algal cell suspensions. In this study, UVA and UVB extinction were determined for cultures of algae with varying cell densities, and the data were used to calculate the corresponding extinction coefficients for both UVA and UVB wavelength ranges. Integrating the Beer–Lambert equation to account for changes in the radiation intensity reaching each depth, from the surface until the bottom of the experimental vessel, we obtained the average UVA and UVB intensity to which the cultured algal cells were exposed. We found that UVR intensity measured at the surface of *Chlamydomonas reinhardtii* cultures lead to a overestimation of the UVR dose received by the algae by 2–40 times. The approach used in this study allowed for a more accurate estimation of UVA and UVB doses.

1. Introduction

1.1. UVR measurement in ecotoxicological studies

Ozone decline during the 1980s and 1990s increased the amount of ultraviolet radiation (UVR) reaching the Earth's surface [1], raising concern about the impacts of UVR on biological systems [2]. Since then, a huge research effort has focused on assessing and predicting UVR impacts on human and natural ecosystems at different biological scales [2].

Because of the importance of algae and macrophyta as primary producers in aquatic environments, the effects of UVR effects on these organisms has been studied extensively [3,4]. In aquatic environments, numerous materials absorb and scatter light, contributing to vertical light attenuation. These include dissolved substances [5,6], particles [7,8] and organisms [9]. Vertical light attenuation processes result in a decrease of the intensity and changes in the light spectrum. While light attenuation is routinely considered in ecological studies of UVR [10], it is not often considered in ecotoxicity studies using algae or other aquatic organisms [11,12]. In such studies, the responses of organisms are generally related to doses of UV that are calculated using measurements of UV intensity at the surface of experimental vessels or liquid media. While UVR extinction may be irrelevant under certain conditions (i.e., low cell densities, low depth of experimental vessels and high media transparency), the cell concentrations required for laboratory studies are expected to significantly attenuate UVR intensity [13,14]. Clearly, in that way, doses received by algae cells during UVR exposure can be overestimated, leading researchers to conclude that the observed effects occur at lower doses than the real ones [15–19].

1.2. Integrative approaches for PAR modelling and UVR extinction assessment

Standardised procedures have been proposed for calculating the vertical attenuation coefficients for photo-synthetically active radiation (PAR) models based on phytoplankton suspensions [13,20]. In the case of UVR, recent studies have demonstrated that simple laboratory measurements allow for establishing reliable relationships between concentrations of optically active substances (such as chlorophyll, dissolved organic matter and total suspended solids) and the underwater UVR light climate in natural systems [10].

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These approaches are based on the theoretical assumption that light passing through a dilute suspension of cells should obey Beer–Lambert's law [10]. Accordingly, optical density is proportionate to the number of cells [21,22]. Nonetheless, other factors, such as cell size, cell shape and intracellular pigment concentration, can modify light attenuation in a cell suspension [13,20]. Even if the major factors that determine light extinction characteristics in cell suspensions are identified, the specific absorption of light is not a linear function of either pigment concentration or cell size [14]. The non-linearity of light absorption results from the "package effect", which represents the decreased light absorption of pigments contained in particles relative to the absorption of the same pigments in solution [13]. Nevertheless, studies of various phytoplankton species have shown significant relationships between cell size and volume and light absorption, indicating that morphological and population parameters may be useful for developing improved models that link biological and optical properties [14]. At present, there is a lack of methodological approaches for laboratory studies that consider UVR extinction.

1.3. Objectives of the study

The goal of this study was to improve the measurement of UVR intensity in algal cultures under agitation (assuming that the cells would be moving throughout the entire medium in the vessel) by calculating UVR extinction coefficients. A spectroradiometer that provides a spectral power distribution (power per unit area per unit wavelength) was used. We also tested a radiometer equipped with simpler UVA and UVB integrative sensors. UVA and UVB extinction were measured using different algal densities of two strains of *Chlamydomonas reinhardtii* and *Synechocystis* sp. to test the robustness of our proposed approach. The extinction coefficients (k) for UVA and UVB were calculated and modelled as a function of OD (the optical density, a proxy for number of cells). We demonstrated that our approach was a rapid method for using light absorption to estimate k_{uva} and k_{uvb} with algal cell suspensions.

2. Materials and methods

2.1. Algal cultures

The experiments were performed in various labs over various time periods, using the cultures available at each lab: *C. reinhardtii*, CC-125 (Chlamydomonas Resource Center, Univ. Minnesota, MN 55108, USA), and 137C+ 83.81 (Institute for Plant Physiology (University of Göttingen, Germany). The two strains of this eukaryote algae showed differences in a few genome sequences [23]. The culture medium was prepared according to the procedures described by Le Faucheur et al. [24]. The algae were grown in a HT Multitron (Infors, Bottimingen, Switzerland) at 25 °C with continuous illumination of 120 μMol photons s^{-1} cm^{-1} (Philips Coolwhite TLD 15 W fluorescent lamps) and shaken at 90 rpm. For cell number and cell volume measurements, a 200 μL sample of the cell suspension was added to a final volume of 4 mL Isoton II diluent solution (Beckman Coulter) and then counted using a Z2 Coulter Particle Counter (Beckman Coulter, Nyon, Switzerland) within the 2.7–10.64-μm size range.

Synechocystis PCC 6803 wild type (Cyanophyta) was obtained from the Pasteur culture collection (Institute Pasteur, France). The culturing procedure was similar to that described for *C. reinhardtii*, but a culture medium (BG-11) was used [25] and the illumination was reduced to approximately 40 μMol photons s^{-1} cm^{-1}. This photosynthetic prokaryote was selected to test the reliability of the experimental approach with smaller sized algae. The word algae will be used through text indistinctly for both the prokaryote and the eukaryote specie.

Experimental batch cultures were prepared by transferring an inoculum of algae in the exponential growth phase at a starting density of approximately 6×10^5 cells mL^{-1}. Cell densities (see details in Table 1) were obtained by centrifuging (3000 rpm, 10 min) the experimental batch cultures and then resuspending the cell pellet until the desired density was reached.

For the estimation of the chlorophyll *a*, algal cells from sampled aliquots were centrifuged at acceleration 16,000*g*, resuspended in 80% cold acetone and incubated for 5 min on ice in the dark. Following another centrifugation step, the supernatants were transferred into a transparent 96-well plate and absorption was measured at wavelengths 750, 663, 647, and 470 nm for calculating concentrations according to a previous development [26].

2.2. UVR intensity measurements

Two different systems were used. The first system consisted of a borosilicate beaker placed under a fluorescent lamp (Arimed B, 40 W; Cosmedico Light, Germany) with an emission maximum between 330 and 350 nm (Fig. 1). Because borosilicate is transparent to UVR radiation, the beaker walls were wrapped with black tape to prevent UVR light from entering from a lateral surface). The spectroradiometer (JAZ-EL 200, Ocean Optics, 830 Douglas Ave., Dunedin, FL, USA) was placed under the beaker and a full spectrum scan was recorded that represented the UVR intensity on the bulk surface. The beaker was then filled step-by-step with the appropriate volume of algal culture (of a certain cell density), such that with each step the culture depth (z) increased by 1 cm. UVR intensity was recorded after a few seconds (3–5 s), as soon as the UVR values had stabilised and prior to sedimentation of the cells. New aliquots were added using a 5 mL pipette, which provided sufficient flow to mix the entire liquid column. This assured a homogeneous light pathway for UVR from the surface of the liquid to the sensor in the bottom of the beaker for each sample. This method had been previously applied successfully to measure irradiance extinction in a volume of water that was too shallow to use a radiometer [10] by placing a "vessel" on top of a sensor and repeatedly refilling the vessel with water.

Each recorded spectrum was integrated at a radiant flux density (W m^{-2}) in the range of UV-B (280–320 nm) and UV-A (320–400 nm) and plotted against z (Fig. 2) using Ocean Optics Spectrasuite™ software. The ranges for energy integration were chosen to fit the ranges of the integrative sensors of Solar Light that were used in the second measuring system.

The second system used to quantify UVR intensity consisted of a PMA2100 Radiometer (Solar Light Co., Oak Line, USA) equipped with two sensors (a UVA-2110 WP sensor integrating energy in the 320–400 nm wavelength range and a UVB-2106 WP sensor integrating energy in the 280–320 nm range). The sensors were placed 55 cm away from a UV lamp equipped with either an Osram HTC400-241 bulb (Fig. 1) or fluorescent tubes (Arcadia D3 Reptile Lamp T5, 39 W, 12% UVB, 30% UVA). The algal suspensions were added to the upper part of a 50-mL Uthermöl chamber fixed over the UVR sensors. The upper part of the chamber was a tube (95 mm in length, 25 mm in diameter) made with plastic that was opaque to UVR wavelengths. A bit of silicon was used around the bottom of the tube where the tube made contact with the glass surface of the sensors to avoid leaching of the cell suspension. UVR intensity measurements were made before sequentially adding 5-mL suspension aliquots to completely fill the column. Each aliquot increased the height of the suspension column by 1 cm, thus allowing UVA and UVB intensity data to be plotted as a function of depth.

Table 1
Details of the six experimental setups (first column). UVR refers to the type of lamp used: *HTC* for halogen and *fluo* for fluorescence tubes; Measu. indicates the device used: *spectro* for the spectroradiometer and *radio* for the integrative radiometer; algae indicates the species; Vessel indicates the type of vessel used for measuring UVA/UVB extinction; *Exp.* indicates the use of different cultures (real replicates) and the sequence in which the experiments were performed; OD_{750} indicates the optical density at 750 nm; and the k_{uva}, k_{uvb} and their respective r^2 values are also shown (k units are cm^{-1}). It has been also shown the chlorophyll *a* concentration for selected experiments, expressed both as chlorophyll per volume (*chl a*) and as chlorophyll per cell (pg chl $cell^{-1}$). These last values have been calculated using the values obtained using the cell coulter (data not shown). The last two columns show the Z values at which 99% of the incident UVA and UVB have been attenuated. The last two rows in the table show the models used for calculating k as a function of OD by the method described in Section 3.2 of the manuscript.

UVR	Measu.	Algae	Vessel	Exp.	OD_{750}	k_{uva}	r^2	k_{uvb}	r^2	pg chl $cell^{-1}$	$Z_{1\%}$ UVA	$Z_{1\%}$ UVB
Fluo	Spectro	CC125	Boro	1	0.5895	1.247	0.87	1.808	0.99	2.04	3.693	2.547
				1	0.3339	0.444	0.98	0.6	0.98	1.44	10.372	7.675
				1	0.1895	0.248	0.98	0.367	0.99	2.77	18.569	12.548
				1	0.1037	0.112	0.9	0.174	0.95	3.02	41.118	26.466
				1	0.0576	0.053	0.74	0.092	0.81	2.24	86.890	50.056
				1	0.0261	0.022	0.32	0.039	0.42	1.44	209.326	118.081
				2	0.6448	1.19	0.99	3.798	0.99	2.18	3.870	1.213
				2	0.4049	0.667	0.99	3.76	0.9	2.46	6.904	1.225
				2	0.2347	0.325	0.98	1.21	0.95	2.51	14.170	3.806
				2	0.1410	0.169	0.96	0.789	0.95	2.03	27.250	5.837
				2	0.0750	0.082	0.86	0.243	0.93	2.97	56.161	18.951
				2	0.0419	0.038	0.65	0.102	0.76	2.18	121.189	45.149
				8	0.2313	0.433	0.99	0.5608	0.99	–	10.635	8.212
				8	0.0911	0.1706	0.96	0.2683	0.98	–	26.994	17.164
				8	0.0505	0.0897	0.9	0.1762	0.97	–	51.340	26.136
				8	0.0218	0.0779	0.83	0.2222	0.97	–	59.116	20.725
Fluo	Radio	CC125	Boro	7	0.2818	0.6393	0.99	0.6767	0.8	–	7.203	6.805
				7	0.2030	0.3923	0.99	0.3545	0.99	–	11.739	12.991
				7	0.1658	0.2332	0.98	0.2047	0.97	–	19.748	22.497
				7	0.1069	0.1124	0.95	0.1076	0.92	–	40.971	42.799
				7	0.1050	0.045	0.76	0.049	0.95	–	102.337	93.983
				7	0.1077	0.0076	0.59	0.0108	0.79	–	605.943	426.405
HTC	Radio	137C	Uterm	3	0.1800	0.2458	0.99	0.2724	0.99	1.32	18.735	16.906
				3	0.2900	0.3419	0.99	0.3934	0.98	1.53	13.469	11.706
				3	0.3500	0.3987	0.99	0.4393	0.99	1.10	11.550	10.483
				4	0.1100	0.1289	0.97	0.1224	0.99	1.03	35.727	37.624
				4	0.2900	0.3199	0.99	0.335	0.99	1.28	14.396	13.747
				4	0.4600	0.4404	0.98	0.5104	0.99	1.19	10.457	9.023
				5	0.2400	0.3531	0.99	0.3836	0.99	1.23	13.042	12.005
				5	0.6700	0.4817	0.9	0.6985	0.98	1.18	9.560	6.593
				5	0.7200	0.4887	0.89	0.7238	0.99	1.15	9.423	6.362
				6	0.5241	0.4809	0.95	0.6092	0.97	–	9.576	7.559
				6	1.0095	0.4771	0.77	0.7099	0.85	–	9.652	6.487
				6	1.2299	0.645	0.79	0.9329	0.89	–	7.140	4.936
Fluo	Spectro	137C	Uterm	9	0.300	0.259	0.99	0.318	0.99	–	17.781	14.482
Fluo	Radio	Synech	Boro	10	0.8219	1.8685	0.99	2.0554	0.99	–	2.465	2.241
				10	0.6123	1.2553	0.99	1.3719	0.99	–	3.669	3.357
				10	0.4302	0.7955	0.99	0.819	0.99	–	5.789	5.623
				10	0.2698	0.3889	0.99	0.5826	0.99	–	11.842	7.905
				10	0.1799	0.2051	0.99	0.3884	0.99	–	22.453	11.857
				10	0.1213	0.0898	0.97	0.2709	0.99	–	51.283	17.000

Models for k_{uva}
$k = 2.01\ OD_{750} - 0.09$ (r^2 0.97)
$k = 0.18\ \ln(OD_{750}) + 0.56$ (r^2 0.91)

Models for UVB
$k = 5.36\ OD_{750} - 0.19$ (r^2 0.70)
$k = 0.31\ \ln(OD_{750}) + 0.79$ (r^2 0.96)

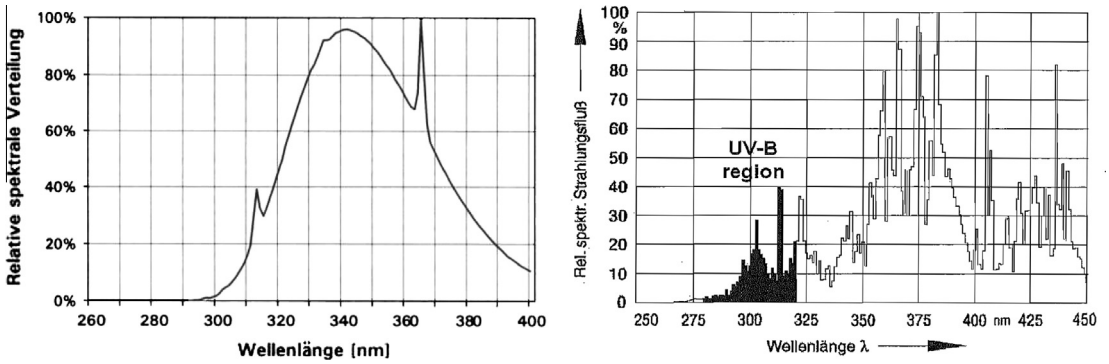

Fig. 1. Distribution of spectral power of the UV lamps used in this study. At left: ARIMED fluorescent lamps (showing the 260–400 nm range). At right: OSRAM HTC bulbs (showing the 250–450 range and the UVB region, in black).

2.3. Experimental design

To test the robustness, feasibility and usefulness of this new methodological approach, various experimental setups that combined different algal strains or species and measuring systems were tested (Table 1). To examine the role of cell density in UVR extinction, three different (i.e., independently developed) cultures of *C. reinhardtii* CC125 at varying cell densities were exposed to

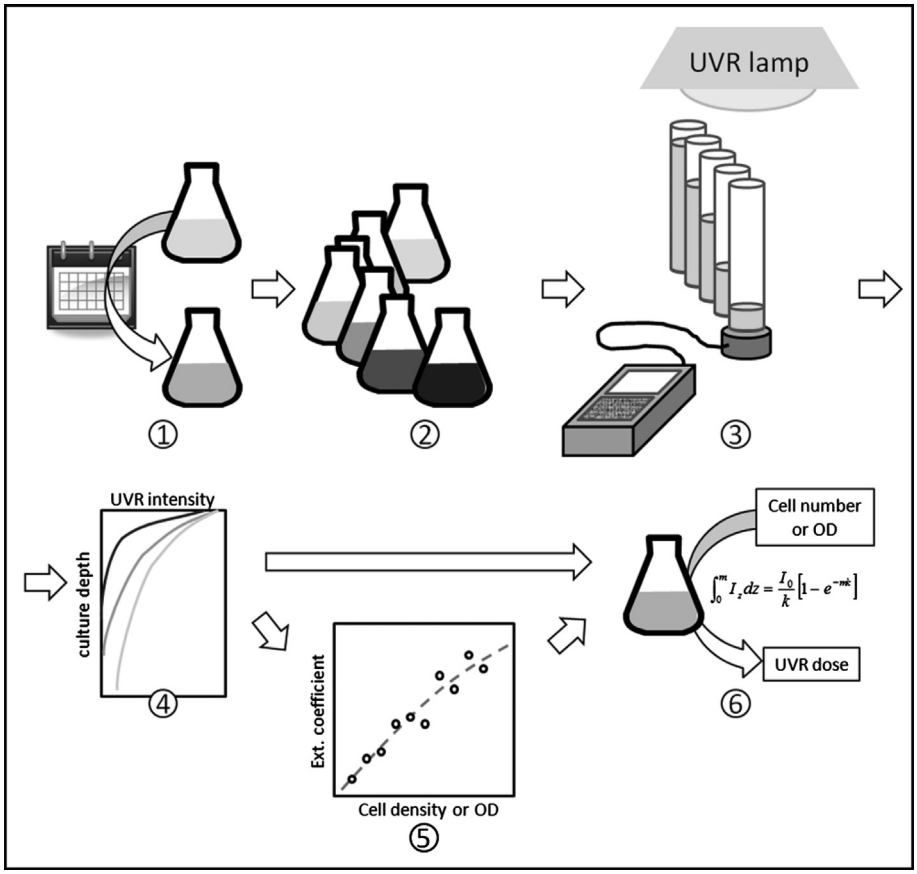

Fig. 2. Diagram showing measurement setup and data processing. The method was applied to standardised algal cultures (1). A dilution bank of the cultures is prepared (2). Extinction coefficients are measured by sequentially adding fixed volumes of each algal dilution to a column exposed to UVR (3). Each time additional volume is added to the column, the depth of the column increases, and UVA and UVB intensity is recorded. The resulting UVR intensity data are plotted against depth and the Beer–Lambert equation is used to obtain the extinction coefficient (k) for UVA and UVB (4). The k values were used to calculate the average intensity of UVR received by a cell suspension (6). It is also possible to plot all k values calculated as a function of the number of cells, or OD (5) and then fit these values to an appropriate model. The model could be used to estimate the k of a given algal suspension.

UVR using the fluorescent tubes previously described. UVR extinction was measured in a borosilicate beaker using a spectroradiometer (Experiments 1, 2 and 8). See details of the whole experimental setup in Fig. 2. To assess the influence of different UV measuring devices on extinction calculations, an integrative radiometer was used (Experiment 7). *C. reinhardtii* (137C + 83.81) was used in Experiments 3, 4, 5 and 6). UVR extinction was measured in a Uthermöl chamber using HTC lamps and the integrative radiometer. The potential effects of using different UVR lamps, measuring devices and vessels were tested in Experiment 9. Finally, to test the influence of smaller-sized algae on UVR extinction, *Synechocystis* PCC 6803 wild type (Institute Pasteur, France) was used (Experiment 10). In this case, UVR extinction was measured in borosilicate beakers using the PMA2100 Radiometer.

2.4. Statistics and modelling

The UVA and UVB extinction data from all experiments were adjusted to the Beer–Lambert equation (Equation 1) using Sigma Plot 12.5 (Systat Software Inc., San Jose, USA). Excel was used for fitting the linear models. To assess the range of cell density for which our approach was most robust, r squared modelling terms were used (see Fig. 3C).

2.5. UVA and UVB extinction coefficients

The coefficients k_{uva} and k_{uvb} were estimated by using the UVA and UVB intensity values from all experiments in the Beer–Lambert equation. All k values are shown in Table 1. Later, the resulting UVA and UVB extinction coefficients were represented as a function of the OD_{750} of the cell suspension (used as a proxy of cell density) and adjusted to a polynomial equation (see details in Fig. 3B). This was designed to eventually allow for the estimation of extinction coefficients based on the OD_{750} of the cell suspensions (details in Section 3.2).

2.6. Calculating average UVA and UVB intensity received by algal cultures

The Beer–Lambert equation, integrated from depth 0 to m (expressed in cm, with m being the total depth of the solution), was used to calculate the average energy received by an algal cell in a cell suspension presenting a certain k_{uva} or k_{uvb} and exposed to I_0 (i.e., UV intensity) at the surface of the liquid (Eq. 2). The resulting value (intensity integration) was divided by depth (expressed in cm to maintain homogeneity in the units). This value (the average UVR intensity received by a cell in the suspension) was multiplied by exposure time to obtain the UVR dose received by the algal cells.

3. Results and discussion

3.1. UVR extinction coefficients

UVB and UVA radiation intensity decreased exponentially with increasing culture depth in all experiments (Fig. 3A), following the Beer–Lamberts equation (Eq. 1). This behaviour was independent

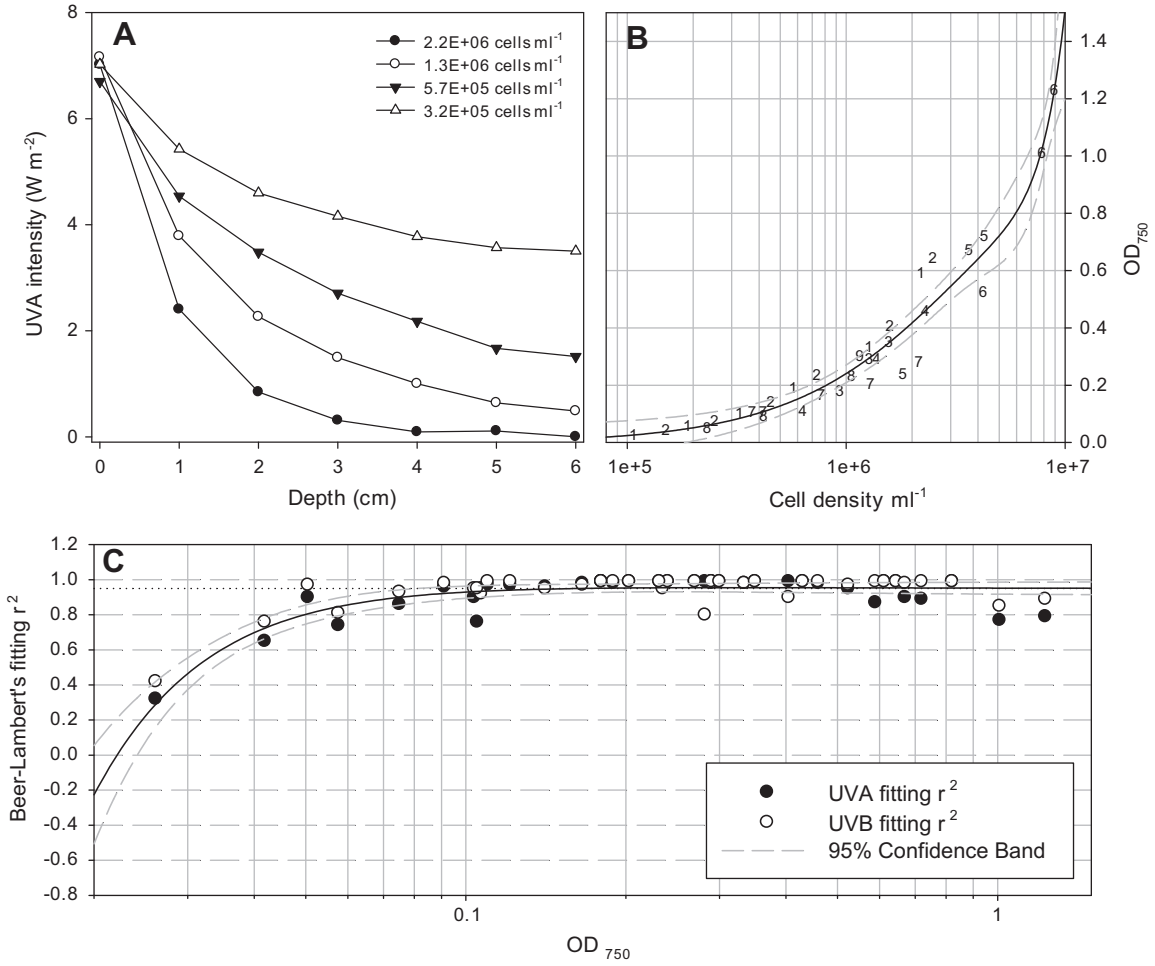

Fig. 3. Graph A shows UVA extinction as a function of depth for four different cell densities. Values at 0 cm represent UVR intensity on the bulk surface. Graph B shows the correlation between OD_{750} and number of cells. The data showed a good fit ($r^2 = 0.94$) to a polynomial cubic equation (OD = −0.0035 + 2.8E−7 cells − 4.2E−14 cells^2 + 2.8-E−21 cells^3). Numbers (1–9) represent the different experiments (see Table 1). The black line represents the fitted model, and the long dashed grey lines are the 95% confidence intervals for that model. Graph C represents the r^2 fitted values of the Beer–Lambert equation for different values of OD (used as a proxy for cell density). The black dotted horizontal line represents $r^2 = 0.95$. White points represent k_{uvb} values and black points represent k_{uva} values, regardless of the device used for measurement. The black line is the adjusted curve ($f_{(x)} = y_0 + (a/x) + (b/x^2)$ with a r^2 of 0.75), with the 95% confidence intervals used as the criteria for selecting the applicability range of the method.

of the device used to measure UVR, cell density or the type of vessel used. The extinction coefficients k_{uva} and k_{uvb} showed a linear relationship with cell density (measured as OD_{750}), as shown in Fig. 4. Accordingly, the highest k_{uva} and k_{uvb} values were obtained for the suspensions with the highest cell density (Table 1).

Values for k_{uva} ranged from 0.0076 to 1.86 cm^{-1}, whereas k_{uvb} ranged from 0.010 to 3.79 cm^{-1}. The higher values of UVB extinction compared to UVA extinction were consistent with the weaker absorption of UVA by biomolecules present in cells [27]. The *k* values determined in our study were in the range of those determined for water with comparable concentrations of chlorophyll *a*. The *k* value for wavelengths under 400 nm was between 1 and 16 m^{-1} [28] for a freshwater lake, between 1 and 2 m^{-1} for water with a chl-a concentration of 0.5–0.6 μg L^{-1} and approximately 1 m^{-1} for water with a chlorophyll a concentration of 0.3 μg L^{-1} [29]. In our study, cell suspensions with chlorophyll concentrations between 0.4–0.8 μg L^{-1} had a k_{uva} between 2 and 4 m^{-1}.

The use of different UVR measuring devices affected the dose calculations, depending on the UV range selected (see Fig. 4). As shown in Fig. 4, the k_{uva} values were quite similar, regardless of the device used. The cultures that clearly differed from the others in terms of UVR dose (Cultures 5 and 6) were those with the highest cell densities. These differences can be attributed to the fact that the sensors on the integrative spectroradiometer only record part of the incident energy (approximately 64%). This effect was even more apparent for UVB radiation than for the UVA range. For these cultures, k_{uvb} was between 30% and 80% higher than k_{uva}. This difference reflected the higher extinction of UVB (i.e., lower penetration in the water column) in the cell suspensions (see *k* values in Fig. 4A1 vs. A2).

Regarding the application of this approach to differently sized algae, Fig. 4 shows that extinction coefficients from Experiment 10 (using *Synechocystis* sp.) shown similar values and trend that other experiments using *Chlamydomonas* and the same measuring setup (Experiment 10). The content of pigment per cell (Table 1) did not shown any kind of influence on the extinction coefficients; correlations resulted in $r^2 = 0.03$ for k_{uva} and 0.01 for k_{uvb}.

For long-term UVR exposure, time-dependent variability in *k* is an important factor to consider. High UVR intensities may cause bleaching of cell pigments or cell death, thereby modifying *k* values. Therefore, when using the proposed method for long-term studies, time-dependent variation in *k* should be assessed and integrated into the calculations. In our case, exposures up to two hours did not modify the *k* values (results not shown).

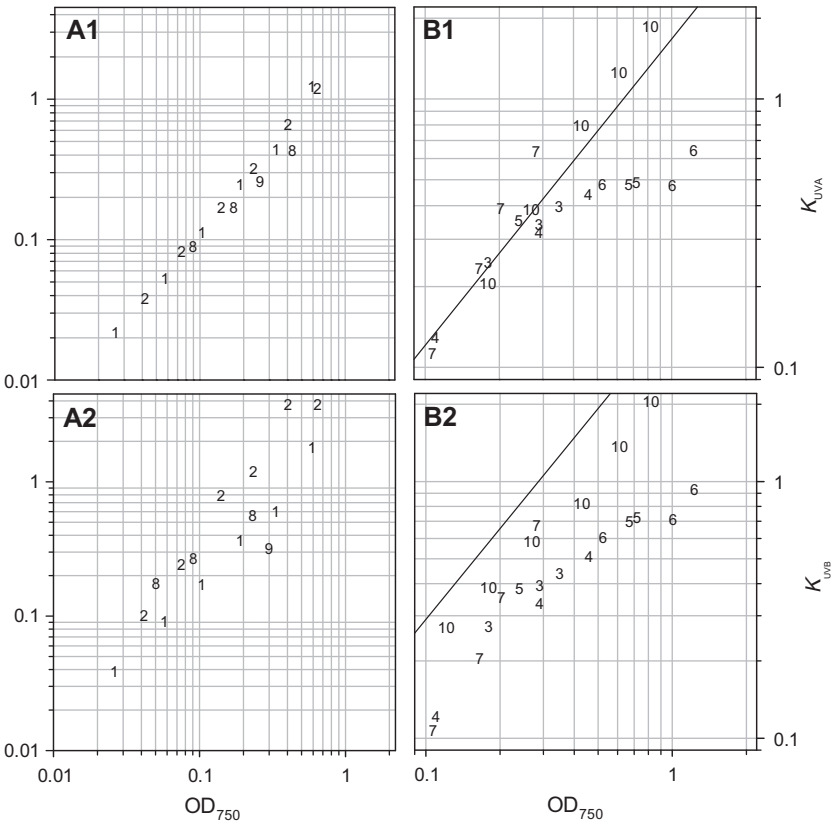

Fig. 4. Extinction coefficients for UVA (A1 and B1) and UVB (A2 and B2) calculated using a spectroradiometer (A1 and A2) or an integrative radiometer (B1 and B2) and represented as a function of the OD. Numbers represent the different experiments (see Table 1). For the purposes of comparison, the linear models of A1 and A2 are shown as lines in B1 and B2.

3.2. Assessing the cell range for which OD750 is a reliable estimator of k_{uva} and k_{uvb}

Experiments focusing on the effects of UVR on algal cultures use homogeneous algal populations that have similar physiological states, pigment contents and size. Based on this practice and the previously described rationale for PAR studies (see Section 1.2), UVR extinction under controlled conditions would vary only with cell density or any of its proxies, such as OD_{750} (see Fig. 4). Therefore, data for k and OD_{750} have been fitted to a polynomial equation (see details in Fig. 3B). Eventually, this model could allow for the estimation of extinction coefficients based on the OD_{750} of a cell suspension.

The r^2 values for all extinction curves were represented as a function of the OD_{750} and fitted to a polynomial inverse second order equation (see details in Fig. 3). This approach identified the OD values for which the experimental data showed a good fit to the Beer–Lambert model. That values ranged from $OD_{750} = 0.07$ (defined the by the intersection of the $r^2 = 0.95$ and the upper confidence interval of the polynomial model) to $OD_{750} = 1$. At these elevated OD, extinction curves start worsening the fitting to Beer–Lambert model. That OD range corresponds to a cell density range of 2.5E5 to 3.2E6 cells mL^{-1} (*C. reinhardtii*). Finally, we noted that the lowest cell densities showed a poor fit to the Beer–Lambert equation (i.e., lower r^2 values). This point will be further discussed in the practical considerations section.

3.3. Comparison of UVR doses calculated using different approaches

We calculated the UVR dose received by a cell suspension as a function of cell density using three methods: (a) measuring the UVR intensity reaching the surface of the cell suspension, (b) calculating k_{uva} and k_{uvb} using a spectroradiometer (more accurate) and (c) calculating k_{uva} and k_{uvb} using a radiometer (less accurate). UVR intensity reaching the cell suspension was 9.54 and 1.56 mW cm^{-2} for UVA and UVB, respectively, for 1 h in a beaker (10 cm depth). For these calculations, we used data from Experiments 1 and 2 (see Table 1 for details), and the doses are shown in Fig. 5. Without considering UVR extinction, UVR dose was not dependent on cell density (black points on Fig. 5A and B). In contrast, UVR dose based on k was dependent on cell density and had lower values. UVA dose was overestimated by 1.4–20 times, depending on the cell density. The overestimation was higher for the highest cell densities. UVB dose was overestimated by 1.8–39 times. Even if the radiometer measurements underestimated UVR dose compared to the spectroradiometer measurements, the values obtained with the radiometer method provided a more realistic estimate of UVR does than the estimates obtained by measuring UVR intensity at the surface of the liquid.

3.4. Practical considerations

At high cell densities, UVB radiation fell below the detection limit at $z > 1$; and the extinction coefficients were calculated using only two data points ($z = 0$ cm and $z = 1$ cm). Accordingly, in these cases almost all UVA and UVB light (99%) was attenuated within the first centimetres ($z < 10$ cm) of the suspensions (see details in Table 1, last two columns). It is thus important to do not use cell densities higher of that leading to the complete UVR attenuation.

Another effect observed with the beakers was that at $z > 4$, UVA and UVB intensities slightly increased. This was due to the "lense effect" of the beaker walls, but could only be observed under very

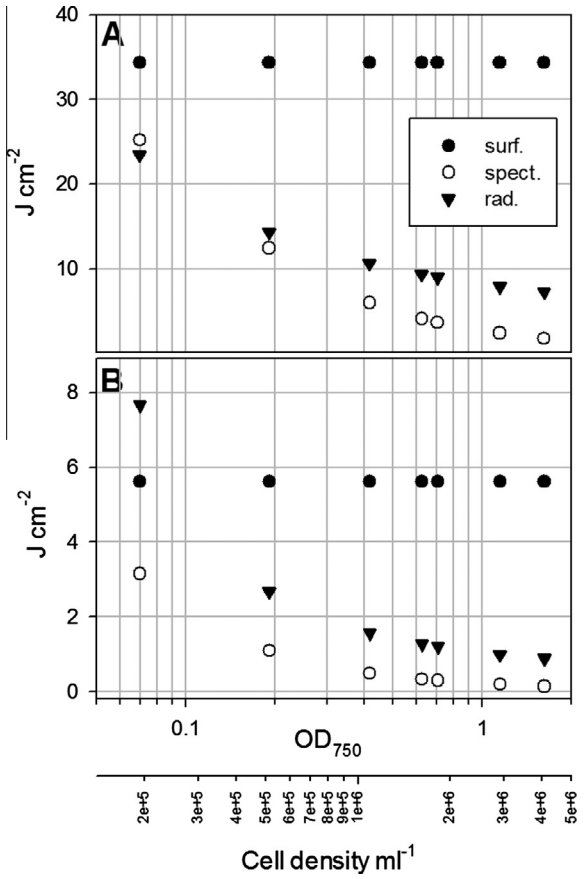

Fig. 5. Comparison of UVA (A) and UVB (B) calculated doses received by algal suspensions of varying cell densities based on different approaches. Surface measurements (surf.) are compared to methods based on k calculations using either a spectroradiometer (spect.) or radiometer (rad.). Results are represented as a function of the OD and cell density.

$$I_z = I_0 \cdot e^{-zk}$$

Eq. 1. Beer–Lambert's equation of light extinction. I_z represents the light intensity at depth z (cm); I_0 is the intensity at the surface of the bulk suspension; and k the extinction coefficient.

$$\begin{aligned} I_z &= I_0 \cdot e^{-zk} \\ \int_0^m I_z &= I_0 \int_0^m e^{-zk} \\ &= \frac{I_0}{-b}\left[e^{-zk}\right]_0^m \\ &= \frac{I_0}{-b}\left[e^{-mk} - e^{-0k}\right] \\ &= \frac{I_0}{-b}\left[e^{-mk} - 1\right] \\ &= \frac{I_0}{b}\left[1 - e^{-mk}\right] \\ \int_0^m I_z dz &= \frac{I_0}{k}\left[1 - e^{-mk}\right] \end{aligned}$$

Eq. 2. The Beer–Lambert equation integrated for depths 0 to m (expressed in cm, with m being the total depth of the solution) to allow for calculating the average energy received by each cell in a given cell suspension with the coefficient k_{uva} or k_{uvb} and a UV intensity I_0 at the surface of the liquid.

transparent media conditions, such as in the samples with very low cell densities. This may also help explain the poor fit of the data to the Beer Lambert equation for these samples. When the beaker was almost full, more light was diverted towards the sensor that, at a lower culture depth, would have been absorbed by the walls of the beaker (or more likely, by the black tape with which the beaker was wrapped). For that reason, some of the data points were excluded from the model fitting. This effect would be most likely be specific to each particular experimental setup, depending on the distance of the lamp from the vessel and the type of vessel used. For this reason, and the differences that may arise from using the different UVR measuring devices in the market, it is always need to calibrate the experimental setup used (see Section 3.1).

The similarity between k values calculated in this study and those measured in real environments showed that our approach provides much more realistic calculations of UVR dose than those based in UVR intensity at the surface of the liquid. Even if the accuracy of k calculations may be improved by considering sensor shape corrections (e.g., the use of common plane sensors vs. the spherical sensors used in open waters), and applying calibrations for the different media encountered by the light pathway from the lamp to the sensor, the differences with our method would be small. Nevertheless, the simplicity and affordability of the our approach, and the robustness (shown using different measuring setups, different algal species and different pigment concentrations) result in more precise UVR dose response calculations, allowing for comparing results among various natural and in vivo studies. This experimental approach would be also useful for other than photosynthetic cell suspensions.

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