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Environmental- and growth stage-related differences in the susceptibility of terrestrial isopods to UV radiation

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ABSTRACT

Global environmental changes are nowadays one of the most important issues affecting terrestrial ecosystems. One of its most significant expressions is the increasing ultraviolet radiation (UVR) arising from the human-induced depletion in ozone layer. Therefore, to investigate the effects of UVR on the terrestrial isopod *Porcellionides pruinosus* a multiple biomarker approach was carried out. Two experiments were performed in order to analyze the importance of the exposure environment and the growth stage on the UV-induced damages. First, adult individuals were exposed to UVR in three exposure environments (soil, soil with leaves, and plaster). Thereafter, three growth stages using soil as the exposure condition were tested. Integrated biomarker responses (IBR) suggested that UV effects were higher in plaster, and mostly identified by changes in acetylcholinesterase and glutathione-*S*-transferases activities, lipid peroxidation rates, and total energy available. The effects in soil and soil with leaves were not so clear. In the growth stages' experiment, juveniles and pre-adults were found to be more affected than adults, with the greatest differences between irradiated and non-irradiated isopods occurring in energy-related parameters. Our findings suggest that soil surface-living macrofauna may be prone to deleterious effects caused by UVR, highlighting the importance of taking the media of exposure and growth stage in account.

1. Introduction

Over the last decades, a growing awareness has emerged concerning the effects of ultraviolet radiation (UVR) in terrestrial ecosystems. The main factor contributing to this concern is the human-induced depletion of stratospheric ozone layer, that is leading to a higher amount of UVR reaching Earth's surface [1]. Notwithstanding the recent efforts to deal with the problem at a global scale, it is unlikely that radiation levels can return to pre-1980 values in the next decades [2,3]. These projections highlight the importance of understanding how this increment in UVR will affect terrestrial biota.

A considerable amount of work was already published concerning the effects of UVR in terrestrial organisms. Nevertheless, much of this work has been focused on plant species [see [4,5], for a review] or vertebrates, mostly in a human health perspective [6–8]. Little attention has been paid to soil invertebrates since they are often assumed to be morphologically well protected and/or able to escape from high intensity radiation [9,10]. However, when analyzing the situation in a long-term perspective, organisms may be unable to cope with the cumulative effects predicted and their defense mechanisms can be overwhelmed [11]. Indeed, several examples of UV-induced injury were already reported in soil biota and a multiplicity of physiological pathways were shown to be affected [12–15]. These effects are thought to be mostly related to the generation of reactive oxygen species (ROS), responsible for oxidative damage in biomolecules [16–18]. When irreversibly damaged, these organisms' cells may undergo apoptosis [14]. Otherwise, damages can be fixed through cells' repairing mechanisms (e.g. glutathione related enzymes) [19], which will also lead to higher energy consumption, that in other conditions would be allocated to other traits, like growth, or reproduction, possibly impairing their ecological function [20]. In the end, such sub-lethal effects can still decrease organisms' performance and might therefore represent strong impairments at the population level, being highly ecologically relevant [16].

Biomarkers have been successfully used to evaluate the effects of sub-lethal levels of a wide range of stressors in an extensive number of different organisms [e.g. [21–25]]. Hence, they are widely acknowledged as a good indication of early signs of stress [26,27], becoming particularly useful with stressors expected to have long-term cumulative effects, which is the case of UVR [11]. Likewise, the measurement of the energy budget is also a valuable tool to have an insight into organisms' condition because it influences all life-history traits [28]. Some attempts have been done recently

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to develop indices that can integrate the overall results of biomarkers. One of them is the integrated biomarker response (IBR) designed by Beliaeff and Burgeot [29]. Originally conceived to optimize the use of biomarkers in field studies, it is also expected to be very useful in laboratory tests. After the transformation of biomarkers' results in a general index value, they may be computed as the area of a star plot, providing an overview of the variations found within the battery of biomarkers under study [29].

In this work we evaluated the effects of UV radiation in *Porcellionides pruinosus*, a widely distributed terrestrial isopod that is considered a key species in edaphic ecosystems because of its involvement on decomposition and nutrient recycling processes [30]. Moreover, it is frequently used in ecotoxicological tests, being described as a good test-species [31].

When assessing the effects of a stressor, one must have into account that organisms' sensitivity may be influenced by several factors, such as their surrounding environment and the growth stage. In order to analyze the relative importance of these factors, we divided our work in two experiments. First we exposed adult individuals of *P. pruinosus* to high doses of UVR in three simulated environments (soil, soil with leaves, and plaster). In the second experiment, we exposed individuals of *P. pruinosus* in three different growth stages (juveniles, pre-adults and adults) to high doses of UV radiation, using soil as an ecological relevant exposure condition.

In order to evaluate if there were differences in the susceptibility of this species to UVR that could be related to the environment surroundings or the growth stage, a battery of biomarkers and measurements of energy reserves was undertaken and plotted in an IBR index.

2. Materials and methods

2.1. Test organisms and soil

The terrestrial isopod *P. pruinosus* was used as test-species. Animals were collected in a horse manure heap and kept in laboratory cultures at 20 °C (±1 °C), 16 h:8 h (light:dark) photoperiod, with soil adjusted to a moisture content of 60% and fed *ad libitum* with alder leaves (*Alnus glutinosa*). Juveniles, pre-adults and adults were considered based on their weight range as 5–10 mg, 10–15 mg and 15–25 mg, respectively. Nevertheless, isopods whose weight was too close to these limits were avoided. Moulting animals or those showing any visible problem (e.g. lack of an antenna, problems in locomotion) were also not used in this study. No sex differentiation was done, but pregnant females were not used.

All tests performed in soil used the certified loamy sand soil LUFA 2.2 (LUFA Speyer). The properties of this soil include a pH = 5.5 ± 0.2 (0.01 M $CaCl_2$), water holding capacity = 41.8 ± 3.0 (g/100 g), organic C = 1.77 ± 0.2 (%), nitrogen = 0.17 ± 0.02, texture = 7.3 ± 1.2 (%) clay; 13.8 ± 2.7 (%) silt and 78.9 ± 3.5 (%) sand.

2.2. UV irradiation

Exposure to UVR took place in a room with controlled temperature and light conditions (20 ± 1 °C and 16 h:8 h, light:dark). UV irradiance was supplied by a UV lamp (Spectroline XX15F/B, Spectronics Corporation, NY, USA, peak emission at 313 nm and 365 nm corresponding to UV-B and UV-A respective peaks) that was placed 30 cm above the boxes containing the isopods. Isopods were simultaneously exposed to UV-A and UV-B radiation. In order to filter UV-C wavelengths, the UV lamp was covered with a clear cellulose acetate film (0.003 mm, Grafix plastics, USA). This cellulose film had been previously irradiated during 12 h to allow the stabilization of radiation intensity passing through it. Isopods were exposed to a single irradiation event with 8 h. The intensity across the radiation spectrum was measured with a spectro-radiometer connected to a monochromator and analyzed with BenWin + software (Bentham Instruments, Reading, UK). UV-A and UV-B average peak intensities in the simulated environments' experiment were 74.46 mW/m^2 nm and 141.14 mW/m^2, respectively, and 44.61 mW/m^2 and 99.21 mW/m^2 nm in the growth stages' experiment. Since the effectiveness of damages to biological tissues varies with the wavelength, intensity values were corrected by using the weighting factors of the CIE reference action spectrum for erythema in human skin [32]. Total biologically effective doses of UVR (UVD_{eff}) used in the simulated environments' and growth stages' experiments were 18.08 kJ/m^2 and 10.3 kJ/m^2, respectively. They were calculated as follows (1), using the biologically effective UV irradiance (I_{eff}) between 280 and 400 nm and integrated into time (2).

$$UVD_{eff}\ (\mathrm{J\ m^{-2}}) = \frac{I_{eff}\ (\mathrm{mW\ cm^{-2}}) \times \text{time of exposure (s)}}{1000} \quad (1)$$

$$\left[UVD_{eff\,(\mathrm{J\ m^{-2}})}\right]_{0h}^{8h} = \frac{UVD_{eff\ 0h} - UVD_{eff\ 8h}}{2} + UVD_{eff\ 8h} \quad (2)$$

2.3. Influence of exposure environment

Isopods were selected from cultures and randomly divided into rectangular plastic boxes (14.3 cm × 9.3 cm × 4.7 cm) with three different substrates (soil, soil with leaves, and plaster), and then exposed to UV radiation. Five replicates were used for each treatment, each one consisting in a box containing twenty isopods. Boxes with the bottom covered with plaster were water saturated overnight prior to the experiment in order to provide isopods an adequate moisture level. Likewise, soil moisture was also adjusted to 60% WHC. Additional water would be added during the course of the experiment whenever necessary. A 35–40% coverage was obtained by including one alder leave on each box of the soil with leaves treatment. After the UV exposure, animals were kept for recovery in soil (60% WHC), and placed inside a climatic chamber at 20 °C (±1 °C), 16 h:8 h (light:dark) photoperiod and supplied with alder leaves. An additional set of 70 unexposed organisms was kept in soil during all the experiment and used as a control. Four isopods per replicate were collected in every sampling time: immediately after the UV exposure (henceforth, T_{Exp}), and after a recovery time of 48 h, 96 h, and 7 days. In all situations, they were individually weighted, freeze-dried in liquid nitrogen to minimize handling-induced effects on the biomarker response and stored at −80 °C until further analysis.

2.4. Influence of growth stage

Isopods of three growth stages (juveniles, pre-adults and adults) were collected from cultures and placed inside circular plastic boxes (Ø 8 cm × 4.5 cm high) with soil adjusted to 60% WHC. Twenty boxes were prepared for each growth stage, each one containing 5 isopods. Ten of these boxes for each growth stage were then submitted to 8 h of UVR whereas the remaining were not exposed and kept as control in a chamber at 20 °C (±1 °C), 16 h:8 h (light:dark) photoperiod. After the UV-exposure, five out of the ten exposed boxes for each growth stage were sampled, along with another five controls, and the remaining were kept for recovery in the control conditions. Food was then supplied in all boxes. After 7 days of recovery, the rest of the boxes (five exposed and five controls) were also sampled. In every sampling time, isopods were collected, individually weighted, freeze-dried in liquid nitrogen, and stored at −80 °C until further analysis.

2.5. Biomarkers analysis

Biomarkers were analysed using the protocol described by Ferreira et al. [27]. For the lipid peroxidation (LPO), glutathione-