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The effects of temperature, soil moisture and UV radiation on biomarkers and energy reserves of the isopod *Porcellionides pruinosus*

Nuno G.C. Ferreira[a,*], Rui G. Morgado[a], António Amaro[a,b], Ana Luísa Machado[a], Amadeu M.V.M. Soares[a], Susana Loureiro[a,*]

[a] Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Department of Environment and Planning & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

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ABSTRACT

Terrestrial isopods from the species *Porcellionides pruinosus* were exposed to different ranges of temperature, soil moisture content and doses of UV radiation. For the temperature and soil moisture content experiments, organisms were sampled after 48 h, 96 h and 14 days of exposure, whereas in the UV experiment, they were sampled at the end of the exposure periods, that consisted on a single-pulse with duration ranging from 30 min to 8 h. For each sampling time the acetylcholinesterase, glutathione *S* transferases, glutathione peroxidase and catalase activities were determined, as well as lipid peroxidation rate. Energy content (lipids, carbohydrates, proteins) and other energy related parameters: energy available, energy consumption and cellular energy allocation were also determined, along with mortality.

The results obtained showed that increases in temperature will affect life traits and specific strategies for isopods to manage their energy budget, in order to handle oxidative stress. It also showed that this species is acclimated to lower moisture scenarios, whereas in case of flood scenarios the turnover point between optimal conditions and mortality is very narrow, which may lead to the local extinction of populations in specific micro-habitats. This study also showed that UV radiation also poses an important stressor for isopods that should be taken in consideration, as the actual doses nowadays present significant negative impact on these organisms.

The study also emphasises that the effects of abiotic factors should be included and taken into consideration by policymakers and that the inclusion of abiotic effects in ecotoxicological tests should be included in the analysis of any stressor to improve chemical risk assessment.

1. Introduction

According to the report "Climate Change 2013: The Physical Science Basis", the climate is changing, with global average air and ocean temperatures increasing, extreme weather conditions being observed more often (including droughts and heavy precipitation), the ultraviolet radiation (UV-R) reaching the planet in higher doses as a consequence of the ozone layer depletion or even the pH of water and soil is being affected (IPCC, 2013).

Such changes constitute an important concern to the scientific community, because they are broad, pervasive and the effects are gradual and hard to detect. The study of these changes in complex environments such as the soil compartment, is of most concern not only due to soil's structure, function, taxonomic diversity and trophic interactions, but also due to the direct and indirect effects on human health and their social and economic activities (Wild, 1993).

In order to investigate related effects of climatic changes in the soil compartment, terrestrial isopods (*Porcellionides pruinosus*) were selected as test-species, because of their key role in soil ecosystems, through the decomposition and fragmentation processes of leaf litter, which may then reflect the effect of stressors on the overall soil functions, causing changes in soil quality and soil services (Drobne, 1997; Lokke and van Gestel, 1998; MEA, 2005). To understand how isopods are being affected by abiotic factors, traditional endpoints like survival, reproduction or feeding rates, may not entirely reflect the effects of these stressors, as they might not detect early effects. For this reason, the endpoints

* Corresponding authors.
E-mail addresses: nunoferreira@ua.pt (N.G.C. Ferreira), ruimorgado@ua.pt (R.G. Morgado), aamaro88@ua.pt (A. Amaro), luisamachado@ua.pt (A.L. Machado), asoares@ua.pt (A.M.V.M. Soares), sloureiro@ua.pt (S. Loureiro).

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chosen to evaluate effects caused by abiotic changes were enzymatic and physiological biomarkers, along with energy related parameters. This choice for biomarkers is mainly due to their sensitivity, quickness and accurate relationship between the stressor exposure and the respective biological response (Morgan et al., 1990). The biomarkers chosen to evaluate the abiotic factors belong to different pathways such as neurotoxicity (acetylcholinesterase), detoxification (glutathione *S*-transferases), oxidative stress (catalase and glutathione peroxidase) and cellular damage (lipid peroxidation). Regarding the measurement of energy related paremeters, they are essential for the organisms life traits such as growth, reproduction, maturation or maintaince, and their depletion affects negatively the population dynamics and structures (de Coen and Janssen, 2003). The measurement of energy reserves as an endpoint has already been used sucessfully in several previous works (e.g. Donker, 1992; Morgado et al., 2013; Stanek et al., 2006), using terrestrial isopods exposed to chemical or abiotic stressors.

Therefore, the main goals of the present study was to simulate changes in abiotic factors that can occur in the soil compartment and determine their effects on biomarkers and energy reserves of the terrestrial isopod *Porcellionides pruinosus*. The abiotic factors used were temperature, soil moisture content, and exposure to an increased dose of UV-R.

The results obtained here will help understanding the processes that terrestrial isopods undergo when exposed to these stressors, how they are coping with them, the stress pathways that will be activated and how the energy budget will be affected. In addition, understanding these changes will also be crucial regarding chemical risk assessment, as changing exposure abiotic scenarios can mislead to a conclusions regarding the response to chemicals by organism. Therefore, results reported here may be included in future studies that will provide input to environmental risk assessment approaches.

2. Materials and methods

2.1. Test organism and culture procedure

The organisms used in this study belong to the species *Porcellionides pruinosus* Brandt (1833), which is considered a synanthropic species and therefore closely related to areas with high human activity. In addition, they are more prone to dig into the soil, being therefore in more close contact to soil particles, than other similar species which live in a closer contact with the litter layer. Organisms were previously collected from a horse manure heap and maintained for several generations in laboratory cultures. In culture, isopods were fed *ad libitum* with alder leaves (*Alnus glutinosa*) and maintained at 22 ± 1 °C, with a 16:8 h (light: dark) photoperiod. Twice a week cultures were water sprayed and food was provided. Only adult animals (15–25 mg wet weight) were used in these experiments and no distinction between sexes was made, although pregnant females were excluded from trials. Animals with abnormalities or under moulting were also excluded.

2.2. Exposures

Tests were performed in plastic boxes (14.3 length × 9.3 width × 4.7 height cm), containing approx. 2 cm of natural certified loamy sand soil LUFA 2.2 (LUFA Speyer) with 12 isopods per test-box. The properties of this soil included a pH = 5.5 ± 0.2 (0.01 M CaCl2), WHC – 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. This is a widely used natural soil for laboratory experiments (e.g. ecotoxicology or ecology) and therefore enables comparison between studies. Organisms were collected from culture boxes, weighted (15–25 mg) and placed in each test-box with alder leaf disks (Ø 10 mm, ± 20 mg) supplied as food. A total of five replicates were used for each exposure scenario and each replicate corresponds to one box with 12 isopods. All previously described conditions were used for the temperature, soil moisture content and UV irradiation exposures.

In temperature and soil moisture content experiments, the exposure lasted for 14 days and biomarkers and energy related parameters were measured after 48 h, 96 h and at the end of the exposure period; in addition organisms collected directly from cultures at the beginning of the exposures were considered as T0. For the UV irradiance experiment, the exposure lasted from 15 min to 480 min (8 h) after which organisms were sampled and biomarkers and energy related parameters were measured. Each

Table 1
Exposure UV doses, after correction for DNA damage (Setlow, 1974) and human skin erythema (McKinlay, 1987) with the corresponding exposure time and peak intensity. Locations where a similar average erythermal UV dose were observed are also presented for ecological relevance (KNMI, 2013). (a)- between December and February, (b)- between March and May, (c)- between June and August, (d)- between September and November. *- Higher UV doses whose correspondence was not found in the literature.

Exposure time (min)	Average peak intensity ($mW\,m^{-2}$)		UV dose (corrected for DNA damage) ($kJ\,m^{-2}$)	UV dose (corrected for Human skin erythema) ($kJ\,m^{-2}$)	Location where dose is considered ecologically relevant
	313 nm	365 nm			
15	113.39	44.11	0.85	0.47	Kingston (Australia)[c]
30	107.21	43.85	1.55	0.86	Sodankyla (Finland)[b]
60	100.45	44.09	2.77	1.66	Angra do Heroísmo (Azores, Portugal)[d]
90	99.78	43.56	4.22	2.48	Sodankyla (Finland)[c]
120	100.49	44.67	5.36	2.98	Brisbane (Australia)[b]
180	97.41	43.70	8.41	4.64	Izana (Tenerife, Spain)[c]
240	103.40	45.10	12.03	6.62	Alice Springs (Australia)[a]
300	105.48	45.25	14.99	8.32	*
360	91.43	42.98	15.34	8.48	
480	116.97	42.35	27.97	15.42	