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In vivo effects of UV radiation on multiple endpoints and expression profiles of DNA repair and heat shock protein (*Hsp*) genes in the cycloid copepod *Paracyclopina nana*

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ABSTRACT

To evaluate the effects of ultraviolet (UV) radiation on energy acquisition and consumption, the copepod *Paracyclopina nana* was irradiated with several doses (0–3 kJ/m^2) of UV. After UV radiation, we measured the re-brooding success, growth pattern of newly hatched nauplii, ingestion rate, and assimilation of diet. In addition, we checked the modulated patterns of DNA repair and heat shock protein (hsp) chaperoning genes of *P. nana*. UV-B radiation induced a significant reduction (7–87%) of the re-brooding rate of ovigerous females, indicating that UV-induced egg sac damage is closely correlated with a reduction in the hatching rate of UV-irradiated ovigerous female offspring. Using chlorophyll *a* and stable carbon isotope incubation experiments, we found a dose-dependent decrease ($P < 0.05$) in food ingestion and the rate of assimilation to the body in response to UV radiation, implying that *P. nana* has an underlying ability to shift its balanced-energy status from growth and reproduction to DNA repair and adaptation. Also, expression of *P. nana* base excision repair (BER)-associated genes and *hsp* chaperoning genes was significantly increased in response to UV radiation in *P. nana*. These findings indicate that even 1 kJ/m^2 of UV radiation induces a reduction in reproduction and growth patterns, alters the physiological balance and inhibits the ability to cope with UV-induced damage in *P. nana*.

1. Introduction

The effects of ultraviolet (UV) radiation on aquatic organisms have been a great concern in recent years as UV radiation negatively influences the relationship of producer (phytoplankton) and consumer (zooplankton etc.,) in the food web, resulting in subsequent effects to the ecosystem and biogeochemical cycles (Häder et al., 2011; McKenzie et al., 2011). UV radiation can directly cause alterations in protein synthesis and DNA through absorption of high-energy photons and can indirectly generate reactive oxygen species (ROS), which cause diverse damage to proteins, nucleic acids, and lipids (Kim et al., 2011; Rhee et al., 2012; Won et al., 2014). In aquatic environments, the detrimental effects of UV radiation on phytoplankton have been extensively studied (Goes et al., 1994; Hessen et al., 1997; Kouwenberg and Lantoine, 2007; Ha et al., 2014a). For example, the effects of UV radiation, such as reduced photosynthesis rate and stimulation of oxygen consumption by respiration, are mainly discussed (Larkum and Wood, 1993; Neale et al., 1998). A low production rate of polyunsaturated fatty acids (PUFA) in UV-irradiated phytoplankton, the primary diet of the gammarid amphipod *Gondogeneia antarctica*, resulted in a reduction in the net production rate of lipid in *G. antarctica*, which then negatively affected its growth and reproduction (Ha et al., 2014a). UV radiation directly and indirectly affects survival, growth, and reproduction in aquatic organisms such as the sea urchin *Paracentrotus lividus*, the rotifer *Brachionus koreanus*, and the copepod *Paracyclopina nana* (Bonaventura et al., 2006; Kim et al., 2011; Won et al., 2014). In particular, zooplankton has received much attention as they have chemical defense compounds (e.g., carotenoid and melanin) and play key roles in transferring essential fatty acids to fish (Borgeraas and Hessen, 2000; Kainz et al., 2004). Also, UV radiation causes increased mortality and damage to eggs, inhibition of hatching, and increased expression of antioxidant enzymes in the copepod *P. nana* (Won et al., 2014).

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In the marine ecosystem, zooplankton plays a crucial role in the aquatic food web, linking producers to higher trophic levels (e.g., macrobenthos or nekton) (Theilacker and Kimball, 1984; Kainz et al., 2004). Of them, the copepod is a dominant group in the marine planktonic system, constituting a large portion of the community and its diversity (Longhurst, 1985). Recently, copepods have been considered a good bioindicator species due to their ease of maintenance under lab conditions and their short life cycle (Raisuddin et al., 2007). Among them, the planktonic copepod *P. nana* is an appropriate ecotoxicological model species as it is distributed in a wide range of estuarine environments, is easily exposed to UV radiation as this it inhabits shallow water, and is susceptible to diverse environmental stressors (e.g., salinity, trace metals, UV- and gamma-radiation) (Hwang et al., 2010; Won et al., 2014; Won and Lee, 2014). Furthermore, its higher susceptibility to environmental stressors, compared to other zooplankton (e.g., heavy metals including Cu and Cd, gamma radiation, UV radiation), makes this species useful as a guideline model species (Lee et al., 2007; Hwang et al., 2010; Rhee et al., 2012; Han et al., 2014a; Won and Lee, 2014).

In this study, we examined the effects of ultraviolet-B (UV-B) radiation (230–360 nm) on reproductive success, transition to copepodite, ingestion of diet, and assimilation rate to evaluate the effects of UV radiation on energy acquisition and its consumption for growth, reproduction, and detoxification. Additionally, modulated expression of the reproductive genes *vitellogenin 1* and *2* (*Vtg1* and *Vtg2*), DNA repair, and chaperoning genes were measured to examine alterations occurring at subcellular levels in response to UV radiation in the copepod *P. nana* as potential bioindicators that alter normal physiology.

2. Material and methods

2.1. Culture of the copepod Paracyclopina nana

The planktonic copepod *P. nana* was collected from Lake Songji (Gangneung, South Korea, 38°20′ 9.89″N, 128°30′ 55.17″E) using a zooplankton net, isolated under a stereomicroscope (SZX-ILLK200, Olympus, Tokyo, Japan), and maintained in the Department of Biological Science, Sungkyunkwan University. The species was identified by mitochondrial DNA cytochrome oxidase 1 (*CO1*) sequence as a barcoding gene (Ki et al., 2009) and by morphological characteristics such as habitus and leg 5 (Ueda et al., 2001). *P. nana* was incubated and maintained in 10 L water tanks with the water adjusted to 15 ppt (Tetramarine Salt Pro®, United Pet Group, Inc., Cincinnati, OH, USA) and pH 7.8–8.2. The tanks were maintained in an automatically controlled incubator (MIR-553, Sanyo, Gunma, Japan) under a 12 h light/12 h dark cycle photoperiod at 25 °C. The oxygen concentration was regulated by aeration to maintain about 7–8 mg O_2/L. The *Tetraselmis suecica* (Chlorophyceae) was supplied as a diet once daily during maintenance, and the seawater was renewed every 3 days.

2.2. UV-B radiation exposure

An ultraviolet (UV) lamp with wavelength of 230 nm to 360 nm (peak 306 nm) was purchased from Sankyo Denki Co., Ltd. (Kanagawa, Japan). The intensity of the UV lamp was measured by a UVX radiometer (Mod M007-043 loaded Mid-Range UVX 300 nm Probe, UVP Ltd., Upland, CA, USA) and regulated by changing the distance between the light and the subject. The exposure doses were controlled by time as follows:

$$\text{Radiation energy(J)} = \text{intensity(W)} \times \text{time(sec)}$$

Exposure intensity (50 μW/cm^2) and doses (1–3 kJ/m^2) were chosen from levels previously described (Won et al., 2014), as those UV-radiation doses induced subcellular oxidative stress and macromolecular alterations in fatty acid composition at a sub-lethal level. For gamma radiation, adult copepods (approximately 300 individuals) were moved to a 50 mL cell culture dish (100 mm × 20 mm, Corning, NY, USA) and were used for each study group (time and dose). For time-dependent analysis of mRNA expression, LD10-24 h levels of UV radiation (3 kJ/m^2) were administered. *P. nana* were sampled at 1, 3, 6, 12, and 24 h after UV irradiation and compared with the non-irradiated group (0 h) as control. During exposure, *P. nana*-containing cell culture dishes were placed in the automatically controlled incubator (25 °C) with a quartz cover (90T1, Taemin Science, Seoul, South Korea) to maintain transparency and to prevent evaporation of seawater (Suppl. Fig. 1 and Suppl. Fig. 2).

2.3. Effects of UV-B irradiation on re-brooding

The female *P. nana* has an unusual reproduction strategy to overcome mate-limited conditions in the natural environment by storing spermatophores in their urosomes (Titelman et al., 2007; Won and Lee, 2014). This reproductive characteristic allows researchers to measure reproductive success using the mated females without males; after the initial hatching or dropping of eggs, the second brooding is measured. To use ovigerous females at the same developmental stage, we collected individuals from the second-generation culture (Suppl. Fig. 1). Briefly, newly hatched nauplii were gathered from ovigerous females (the first generation) using 120 μm sieves and harvested for about 2 weeks, following the maintenance protocol. Then, ovigerous females, having new eggs, were checked every 24 h to gather healthy individuals in the same developmental stage (the second generation) to use in the UV radiation test. Ten ovigerous females were gathered, in triplicate, using a 200 μm sieve and were transferred to four different cell culture dishes (0–3 kJ/m^2) for UV radiation. After that, their re-brooding was measured for 6 days. We counted individuals that had successive new egg sacs following the first hatching or dropping of eggs after UV-B radiation (Won and Lee, 2014).

2.4. UV-B effect on molting

To measure growth success as a chronic assay, the just-hatched *P. nana* nauplii were gathered within 6 h after UV radiation (24, 21, 12, and 12 individuals for each irradiated group from 0 to 3 kJ/m^2) from ovigerous females using a Pasteur pipette and were transferred to a 12-well culture plate (SPL Life Science Co., Pocheon, South Korea) in 3 mL of seawater (1 individual/3 mL). In this study, the *P. nana* nauplii (N1 stage) were exposed to UV radiation during embryogenesis to examine the growth pattern as *P. nana* is an egg-bearing copepod and requires about 24 h for hatching, as mentioned in Fig. 3A and Suppl. Fig. 1. After UV radiation, the nauplii were fed for 20 days by adding 10 μL of *T. suecica* (approximately 200,000 cells/mL). The culture condition was maintained at 25 °C, 15 ppt and renewed every 2 days. Their growth from stage N1 to stages C1 and C4 was measured using a stereomicroscope (SZX-ILLK200, Olympus, Tokyo, Japan) for 20 days following guideline in Han et al. (2014a).

2.5. Ingestion and assimilation rate of UV-B-irradiated copepods using chlorophyll a concentration and ^{13}C-labelled diet

The concentration of chlorophyll *a* and ^{13}C-labeled carbon contents in the phytoplankton *T. suecica* were used as indices for ingestion and assimilation by UV-B-irradiated *P. nana* (Suppl. Fig. 2). The phytoplankton *T. suecica* was reared in autoclaved and filtered seawater enriched with Conway medium (Walne, 1974) in a 20 L vessel under 24 h-light condition. To adjust the isotopic ratio of ^{13}C tracer in the *T. suecica*, the ^{13}C level of the dissolved inorganic