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Effects of UV radiation on hatching, lipid peroxidation, and fatty acid composition in the copepod *Paracyclopina nana*

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ABSTRACT

To evaluate the effects of UV radiation on the reproductive physiology and macromolecules in marine zooplankton, several doses of UV radiation were used to treat the copepod *Paracyclopina nana,* and we analyzed in vivo endpoints of their life cycle such as mortality and reproductive parameters with in vitro biochemical biomarkers such as reactive oxygen species (ROS), the modulated enzyme activity of glutathione *S*-transferase (GST) and superoxide dismutase (SOD), and the production of a byproduct of peroxidation (e.g. malonedialdehyde, MDA). After UV radiation, the survival rate of *P. nana* was significantly reduced. Also, egg sac damage and a reduction in the hatching rate of offspring were observed in UV-irradiated ovigerous females. According to the assessed biochemical parameters, we found dose-dependent increases in ROS levels and high levels of the lipid peroxidation decomposition product by 2 kJ m^{-2}, implying that *P. nana* was under off-balanced status by oxidative stress-mediated cellular damage. Antioxidant enzyme activities of GST and SOD increased over different doses of UV radiation. To measure UV-induced lipid peroxidation, we found a slight reduction in the composition of essential fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These findings indicate that UV radiation can induce oxidative stress-triggered lipid peroxidation with modulation of antioxidant enzyme activity, leading to a significant effect on mortality and reproductive physiology (e.g. fecundity). These results demonstrate the involvement of UV radiation on essential fatty acids and its susceptibility to UV radiation in the copepod *P. nana* compared to other species.

1. Introduction

The UV radiation, reaching the earth's surface, significantly influences aquatic ecosystems and triggers a variety of biological effects. The effects of UV radiation on organisms are a specific concern due to stratospheric ozone depletion (Allen et al., 1998; Häder et al., 2011; Mckenzie et al., 2011). To date, several studies have reported that UV radiation significantly affects survival, growth, immune system, and reproduction in aquatic organisms including fish, sea urchin, and crab (Gouveia et al., 2005; Bonaventura et al., 2006; Sharma et al., 2010). Particularly, in the Arctic environment, several researchers have provided evidence that UV radiation influences physiological conditions, leading to changes in the community composition and distribution of species (Bothwell et al., 1993; Hessen, 2002; Häder, 1994). However, these effects are not limited to Arctic conditions. For example, Zepp (2003) showed that solar radiation is a significant stressor of coral assemblages in tropical and subtropical marine environments. Also, Dahms and Lee (2010) provided a critical review of the effects of UV radiation on marine ectoderms, emphasizing the molecular alterations and physiological responses. Radiation-mediated oxidative stress, lipid peroxidation, and DNA strand breakage are important contributors to the harmful effects on the marine ecosystem (Dahms and Lee, 2010). UV radiation has several repercussions on the level of molecular alterations to community changes (Robinson et al., 2005; MacFadyen et al., 2004; Rastogi et al., 2010). In addition, UV radiation generates reactive oxygen species (ROS) and free radicals via photosensitizing agents (Polle et al., 1993).

In marine ecosystems, copepods a dominant group of the mesozooplankton community in aquatic ecosystems (Longhurst, 1985), play a key role in the fundamental food web, as copepod's nauplii, copepodites, and adults are the main prey for larvae of ecologically and economically important fish species (Theilacker and Kimball, 1984). For this reason, they affect the community structure of phytoplankton as predators. For example, copepod grazing impacts phytoplankton communities (Båmstedt et al., 1992) and copepods directly affect the growth of phytoplankton by sequestering nutrients from the water column (Johnson and Leucke, 2012). Also, copepods are susceptible to diverse xenobiotic factors and have the potential to be a good indicator species

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of the aquatic ecosystem. However, only a little attention has been given to the effects of UV radiation on in vivo and in vitro endpoints of marine copepods. Recently, Rautio and Tartarotti (2010) reviewed the literature for responses to UV radiation on damages and recovery mechanisms in freshwater zooplankton. However, the combinational data on in vivo and in vitro endpoints is required due to a range of variable response at multiple levels of macromolecules to individuals in copepods.

The copepod *Paracyclopina nana* Smirnov, 1935 (Copepod, Cyclopoida) would be one of the most appropriate environmental monitoring species of copepods, as it is widely distributed in estuaries and is tolerant to general environmental conditions such as salinity and temperature, and has a short life cycle (less than two weeks) (Lee et al., 2006). Thus, this species can be a useful model species to explore diverse endpoints during whole life cycles. Also, *P. nana* can be environmentally realistic be exposed to natural UV radiation since this species lives in shallow water.

In this study, we irradiated the marine zooplankton *P. nana* to UV radiations in order to assess the physiological effects on in vivo endpoints (e.g. survival rate, egg damage, and fecundity) and to evaluate in vitro endpoints (e.g. ROS level, antioxidant-related enzymes [glutathione *S*-transferase (GST) and superoxide dismutase (SOD)], and lipid peroxidation) under oxidative stress conditions induced by UV exposure. Also, we examined changes in fatty acid composition in UV-irradiated *P. nana* to analyze how UV radiation affects fatty acid composition and how such parameters can be linked to macromolecular and in vivo endpoints with other measured enzymatic biomarkers.

2. Materials and methods

2.1. Culture of the copepod, Paracyclopina nana

The copepod *P. nana* was collected from Lake Songji (38°20′ 9.89″N, 128°30′ 55.17″E), Gangneung in South Korea and maintained in an aquaculture facility at the Department of Biological Sciences, Sungkyunkwan University. The species was identified by morphological characteristics and by sequence analysis of mitochondrial DNA cytochrome oxidase 1 (*CO1*) as the barcoding gene (Ki et al., 2009). For the experiment, *P. nana* was maintained and incubated in a controlled incubator under a 12 h light and 12 h dark cycle photoperiod at 25 °C. The water condition was adjusted to 15 psu (Tetramarine Salt Pro®, United Pet Group, Inc., Cincinnati, OH, USA) and a pH of about 7.8 to 8.2. A diet of the green microalgae *Tetraselmis suecica* was fed to the *P. nana* once daily.

2.2. UV radiation and its effects on mortality

UV lamps capable of emitting between 280 nm and 360 nm (peak 306 nm) were purchased from Sankyo Denki (Sankyo Denki Co., Ltd., Kanagawa, Japan). UV intensity was measured by UVX radiometer (Model M007-043 loaded Mid-Range UVX 300 nm Probe, UVP Ltd., Upland, CA, USA). To obtain the value of dose response about the UV radiation effect, *P. nana* were irradiated over a wide range of UV radiation (0 to 30 kJ m^{-2}) without visible ray in a UV lamp-installed incubator under 25 °C. The intensity of UV lamp was 50 μW/cm^2 and the irradiated energy was controlled with exposure time using following equation;

$$\text{Joule} = \text{Watt} \times \text{Seconds}.$$

During the radiation, a quartz cover (90T1, Taemin Science, Seoul, South Korea) was used to allow UV transparency and to prevent evaporation of the working seawater. Ten ovigerous *P. nana* females were irradiated with each dose of UV radiation and kept at 25 °C in dark conditions to exclude potential photo-remediation by photoreactivation with photolyase (Sinham and Häder, 2002). The observations were taken at 0, 24, and 48 h after radiation under a stereomicroscope to determine whether they were alive. The tests were run with triplicate for on batch experiment.

2.3. UV radiation effects on eggs and reproduction

To measure dysfunction in reproduction and hatching, the dropped egg clutches were counted and the number of just-hatched nauplii was checked from ovigerous *P. nana* females. The morphology over developmental stages follows the criteria as described in previous studies (Lee et al., 2006; Won and Lee, 2014). For measuring the reproduction rate, 10 ovigerous *P. nana* females were randomly collected using a 200-μm sieve, and the number of dropped egg sacs was counted for 48 h (at 0, 1, 6, 12, 24, and 48 h) after exposure to 1, 2, 3, 4, and 6 kJ m^{-2} of UV radiation. To count the nauplius, we collected just-hatched nauplii to new plates and counted them over six-days under a stereomicroscope. Each result is expressed as percent of damaged eggs and average number of nauplii per one individual.

2.4. Measurement of reactive oxygen species (ROS) and activity of glutathione S-transferase (GST) and superoxide dismutase (SOD)

To assess the cellular oxidative status, we measured reactive oxygen species (ROS). The ROS level was measured using dichlorofluorescein diacetate (DCFDA) oxidized by ROS into 2′,7′-dichlorofluorescein (DCF), a highly fluorescent compound. Briefly, UV-irradiated *P. nana* were gathered at post 1 h of exposure and homogenized with a teflon pestle in a buffer containing 0.32 mM sucrose, 20 mM HEPES, 1 mM $MgCl_2$, and 0.4 mM PMSF (pH 7.4). To remove the debris, the homogenized samples were centrifuged at 10,000 *g* for 20 min (4 °C). The supernatant was reacted with H_2DCFDA and measured at 485 nm for excitation and at 520 nm for emission (Thermo Scientific Varioskan Flash, Vantaa, Finland).

The activity of GST (EC 2.5.1.18) was measured as described by Regoli et al. (1997) with minor modifications. Using a spectrophotometric method, GST activity was calculated by measuring the reduced absorbance of glutathione by conjugation with 1-chloro-2,4-dinitrobenzene at 340 nm. The enzyme concentrations of SOD (EC 1.15.1.1) were measured using a kit from Sigma-Aldrich Co. (St Louis, MO, USA). The relative SOD concentrations were calculated from the reduced absorbance of formazan product (at 440 nm) by the SOD enzyme. The ROS contents and the activity of GST and SOD were normalized to the total protein content by following the Bradford (1976) method, using bovine serum albumin as a standard.

2.5. Lipid peroxidation

The lipid peroxidation was determined by the level of malondialdehyde (MDA), the end product of lipid peroxidation. A lipid peroxidation assay kit (Sigma-Aldrich Co., Saint Louis, MO, USA) and UV-radiated homogenized samples were used according to the manufacturer's instructions. Briefly, thiobarbituric acid (TBA) solution was added into each sample to form the MDA–TBA adduct. This fluorometric product was measured at 532 nm for excitation and 553 nm for emission (Thermo Scientific Co., Varioscan Flash). The level of lipid peroxidation was also normalized to the total protein content as described in Section 2.4.

2.6. Fatty acid composition

Adult *P. nana* (~400 individuals) were irradiated with four different doses of UV (0, 1, 2, and 3 kJ m^{-2}). To secure the time for changing of macromolecules, *P. nana* were gathered at 48 h after UV radiation and freeze-dried. The lipids were extracted with dichloromethane/methanol 2:1 (v/v) after adding internal standard (C19:0) according to the methods described in Hama and Handa (1987), with minor modifications. Extractions were repeated three times with sonification, and the extracted lipids in the dichloromethane phase were separated from the water–methanol phase. The fatty acid methyl esters were prepared by saponification using 0.5 M KOH-methanol, followed by methylation with BF3-methanol.