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# Different susceptibilities of the Antarctic and temperate copepods *Tigriopus kingsejongensis* and *T. japonicus* to ultraviolet (UV) radiation

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ABSTRACT: To understand the effects of UV radiation on the Antarctic copepod *Tigriopus kingsejongensis* and the temperate copepod *Tigriopus japonicus*, the 96 h half lethal dose (LD50-96h) was calculated and compared with the levels of intracellular reactive oxygen species (ROS), antioxidant enzymatic activities, and gene expression profiles of the defensome in response to UV radiation over time (control, 1, 3, 6 h) in these copepods. 'Defensome' refers to the integrated system of defense mechanisms — such as detoxification, antioxidation, apoptosis, and cell proliferation — that were altered by UV exposure. The LD50-96h and no observed effect level (NOEL) at 96 h after UV exposure were determined as 23.16 kJ $m^{-2}$ and 12 kJ $m^{-2}$, respectively, in *T. kingsejongensis* and 26.42 kJ $m^{-2}$ and 12 kJ $m^{-2}$, respectively, in *T. japonicus*. ROS levels in response to 12 kJ $m^{-2}$ UV increased slightly ($p < 0.05$) in *T. kingsejongensis* over time, and were also higher ($p < 0.05$) in *T. japonicus*. Transcript levels of antioxidant-related genes were mostly down-regulated in response to 12 kJ $m^{-2}$ UV radiation, except for glutathione-S transferase delta epsilon (*GST-Delta-E*), manganese-superoxide dismutase (*Mn-SOD*), glutathione reductase (*GR*), and glutathione peroxidase (*GPx*) genes in *T. kingsejongensis*. *T. japonicus* heat shock protein (*hsp*) genes were mostly up-regulated, but only small *hsp* genes (*hsp10* and *hsp20*) showed up-regulation in *T. kingsejongensis*. This finding provides a better understanding of how UV radiation affects *in vivo* endpoints and the relevant molecular response in 2 different copepod species from contrasting environments.

KEY WORDS: Ultraviolet radiation · Antarctic copepod · Temperate copepod · *Tigriopus kingsejongensis* · *Tigriopus japonicus* · Reactive oxygen species



## INTRODUCTION

Ultraviolet (UV) radiation has been of great concern as one of the environmental stressors in aquatic ecosystems, as UV radiation reaching the Earth's surface is increasing due to the loss of stratospheric ozone (Allen et al. 1998, Newman et al. 2006, Dahms & Lee 2010, Mckenzie et al. 2011). Antarctic marine organisms in particular can be affected by UV radiation and temperature (Lu & Wu 2005, Bonaventura et al. 2006, Schwerin et al. 2009, Ha et al. 2014), but it has been difficult to compare species in the same genus from Antarctic and temperate regions for 3 reasons: (1) Antarctic species are very difficult to collect from the natural habitat; (2) molecular identification to confirm the genus of Antarctic and temperate species by mitochondrial analysis is a difficult task; and (3) providing an optimal condition for breeding in the laboratory is difficult.

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In the marine ecosystem, copepods are widely distributed and are ecologically important organisms in the food web, transferring energy between producers and consumers (Theilacker & Kimball 1984, Raisuddin et al. 2007). The copepod *Tigriopus japonicus* (Copepoda: Harpacticoida: Harpacticidae) has been considered a suitable experimental model species for marine ecotoxicology and environmental genomic studies because of its small size (less than 1 mm; Fig. 1), sexual dimorphism, high fecundity, short reproduction period (approximately 2 wk), short lifespan (Raisuddin et al. 2007), and the availability of whole transcriptome information (H. S. Kim et al. 2015). The Antarctic copepod *Tigriopus kingsejongensis* (Fig. 1) was newly introduced as a promising model species with whole transcriptome information for Antarctic ecophysiology and environmental research (Park et al. 2014, Kim et al. 2016, Lee et al. 2016) (Fig. 1). This will be a real asset for ecophysiological and ecotoxicological comparative studies of these species.

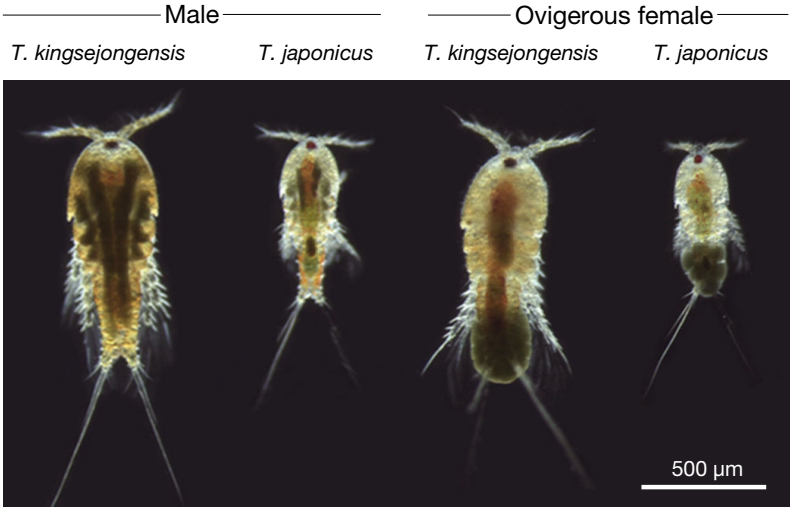


Fig. 1. External morphology of the copepods *Tigriopus kingsejongensis* and *T. japonicus*

Generally, UV radiation can directly lead to DNA damage and also indirectly induce cellular damage through alterations of macromolecules (e.g. proteins, lipids) and by generating reactive oxygen species (ROS) (Sinha & Häder 2002). Detrimental effects of UV radiation (e.g. on survival, growth, reproduction, and behavior) under normal physiological conditions have been reported in diverse aquatic invertebrates, including the crab *Chasmagnathus granulata* (Gouveia et al. 2005) and the sea urchin *Paracentrotus lividus* (Bonaventura et al. 2006). For example, in the sea urchin *Sterechinus neumayeri,* UV-exposure led to adverse effects on embryos and larval development, resulting in increased mortality (Lesser et al. 2004). In the rotifer *Brachionus koreanus* (Kim et al. 2011), the copepod *T. japonicus* (B. M. Kim et al. 2015), and the copepod *Paracyclopina nana* (Won et al. 2014), UV exposure had adverse effects on cellular ROS levels and antioxidant enzymatic activities (e.g. glutathione *S*-transferase [GST], glutathione reductase [GR], superoxide dismutase [SOD], and glutathione peroxidase [GPx]) with transcriptional regulation of antioxidant defense mechanism-related genes (e.g. *GST*, *GR*, *SOD*). In addition, heat shock protein genes (*hsp* genes) are well known as molecular chaperone proteins for cellular protection (through protein folding/unfolding) from DNA damage induced by environmental stressors (Feder & Hofmann 1999, Sarkar 2006). *Hsp* genes were modulated in response to UV radiation in the rotifer *B. koreanus* and in the copepod *P. nana* (Kim et al. 2011, Won et al. 2014). Also, the possibility of *hsp* as potential biomarkers of UV radiation and environmental toxicants in marine copepods has been suggested (Rhee et al. 2009, Lauritano et al. 2012b). Thus, transcriptional regulation of *hsp* genes provides valuable clues about how marine organisms regulate antioxidant and stress responses to UV radiation. So far, however, there is limited evidence of adverse effects of *in vivo* and *in vitro* endpoints in response to UV radiation in Antarctic marine organisms, despite increasing threats to the Antarctic marine ecosystem from rising UV radiation levels and climate change in recent years (Dahms et al. 2011, Häder et al. 2011, Mckenzie et al. 2011). Thus, efforts to understand the underlying molecular response to UV radiation in Antarctic marine invertebrates are crucial to uncover their defense mechanisms in response to environmental stressors in Polar regions.

In this study, to examine different susceptibilities of the copepod *Tigriopus* in Antarctic and temperate regions to UV radiation, we compared the 96 h-half lethal UV dose (LD50-96h) with no observed effect level (NOEL) cellular responses in ROS levels, antioxidant enzymatic activities, and mRNA expressions of antioxidant and chaperone-related genes over time. This study will be helpful for a better understanding of how these 2 congeneric species of Antarctic and temperate copepods mediate ecophysiological and ecotoxicological effects in response to UV radiation.

## MATERIALS AND METHODS

### Culture and maintenance of copepods

The Antarctic copepod *Tigriopus kingsejongensis* (kindly provided by Dr. Sanghee Kim, Korea Polar Research Institute) were maintained at the Department of Biological Science, Sungkyunkwan University. *T. kingsejongensis* and *T. japonicus* were reared and maintained at 32 practical salinity units (psu) in filtered artificial seawater (Tetra Marine Salt Pro) under a 12:12 h light and dark cycle at 15°C for *T. kingsejongensis* and 25°C for *T. japonicus*. Marine microalga *Tetraselmis suecica* were provided as a live diet.

### Acute toxicity in response to UV radiation

To determine the half lethal dose value at 96 h (i.e. LD50-96h), we conducted acute toxicity tests using ovigerous female *T. kingsejongensis* with various doses (0, 12, 15, 18, 21, 24, 27, 30, 33, and 36 kJ $m^{-2}$) of UV (280 to 360 nm) radiation at an intensity of 0.5 W $m^{-2}$ (radiation energy [J] = intensity [W] × time [s]). Ten ovigerous females in triplicate were transferred to a 12-well tissue culture test plate (30012, SPL Life Science) containing 4 ml of artificial seawater, and then the copepods were exposed to UV radiation using a UV lamp (G15T82, Sankyo Denki; wavelength range 280 to 360 nm) with a cover slip of quartz glass (90T1, Taemin Science) to minimize evaporation. The intensity of UV radiation was determined using a UVX radiometer (CON-TROL CURE UV sensor model M007-043, loaded mid-range UVX 300 nm probe model M007-045, UV Process Supply). Subsequently, mortality was checked by counting the number of dead *T. kingsejongensis* under a stereomicroscope every 24 h until 96 h after UV radiation. NOEL and LD50 values were calculated by Dunnett's test and Probit analysis (ToxRat Solutions).

### Measurement of reactive oxygen species (ROS) levels

To determine the status of oxidative stress in the UV-exposed copepods, *T. kingsejongensis* (approximately 30 adults) and *T. japonicus* (approximately 300 adults) were exposed to a NOEL of 12 kJ $m^{-2}$ UV radiation and sampled at 0 (control), 1, 3, and 6 h, and then ROS levels were measured by the cell-permeant 2′, 7′-dichlorodihydrofluorescein diacetate ($H_2$DCFDA) fluorescence method (Kim et al. 2011). Briefly, samples were homogenized in a buffer (0.32 mM sucrose, 20 mM HEPES, 1 mM $MgCl_2$, and 0.4 mM PMSF at pH 7.4) using a Teflon pestle. The homogenized samples were centrifuged at 13 200 × *g* for 15 min (4°C) and the supernatants were reacted with $H_2$DCFDA. Fluorescence peaks were measured at 485 nm for excitation and 520 nm for emission using fluorescence spectroscopy (Varioscan Flash, Thermo Scientific). Total protein content of the supernatant was determined to normalize ROS contents using the Bradford method (Bradford 1976).

### Measurement of antioxidant-related enzymatic activities

To examine the antioxidant defense mechanisms in response to UV radiation-induced oxidative stress, we measured the glutathione (GSH) contents and antioxidant enzymatic activities at 12 kJ $m^{-2}$ (0.5 W $m^{-2}$intensity) in UV exposed adult *T. kingsejongensis* (approximately 30 individuals) and *T. japonicus* (approximately 300 individuals) over time, at 0 (control), 1, 3, and 6 h. Three replicates were measured for each treatment group. The overall experiments were conducted as shown in Kim et al. (2011). The activities of glutathione reductase (GR, EC 1.8.17) and superoxide dismutase (SOD, EC 1.11.1.9) were measured using the GR assay kit and SOD assay kit (Sigma–Aldrich), respectively. The activities of each antioxidant enzyme were calculated by measuring the reduction in absorbance relative to the control using a spectrophotometer (Ultrospec 2100 Pro, Amersham Biosciences). The total protein content was determined to normalize the GR and SOD activities in a dye-binding method using bovine serum albumin as its standard (Bradford 1976).

### Expression of antioxidant and chaperone genes in response to UV

To measure the expression patterns of antioxidant- and chaperone-related genes, we examined mRNA expression profiles of those genes in response to 12 kJ $m^{-2}$ UV radiation over time (0, 1, 3, and 6 h). Total RNA was extracted using TRIZOL® reagent (Invitrogen) according to the manufacturer's instructions. The quantity and quality of total RNA were checked at 230, 260, and 280 nm using a spectrophotometer (Ultrospec 2100pro, Amersham Bioscience). To synthesise cDNA for real-time reverse transcription PCR (RT-PCR), 2 µg of total RNA and

oligo(dT)$_{20}$ primer were used for reverse transcription (SuperScript™ II RT kit, Invitrogen). Real-time RT-PCR was conducted under the following conditions: 95°C for 4 min; 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s; and 72°C for 10 min using SYBR Green fluorescence as a probe (Molecular Probe) with a CFX96™ real-time PCR system (Bio-Rad). To confirm amplification of specific products, melting curve cycles were run under the following conditions: 95°C for 1 min, 55°C for 1 min, 80 cycles of 55°C for 10 s with 0.5°C increase per cycle using real-time RT-PCR F or R primers (Table 1). The *T. kingsejongensis* and *T. japonicus 18S rRNA* genes were used to normalize expression levels between samples. All experiments were performed in triplicate. The relative fold change of the gene expression was calculated by the $2^{-\Delta\Delta C_T}$ comparative method (Livak & Schmittgen 2001).

### Statistical analysis

All the results are expressed as mean values. The normal distribution and homogeneity of variances of data were checked by Levene's test. Data were analyzed by using 1-way ANOVA followed by Tukey's honestly significant difference test ($p < 0.05$). All the statistical analyses were performed using SPSS® version 21 software.

## RESULTS

### Acute toxicity in response to UV radiation

Acute toxicities of UV radiation to ovigerous female *Tigriopus kingsejongensis* and *T. japonicus* were measured 96 h after UV radiation with increasing doses (0, 12, 15, 18, 21, 24, 27, 30, 33 and 36 kJ m$^{-2}$). Mortality increased significantly ($p < 0.05$) with increasing UV dose. LD50-96h and NOEL of UV radia-

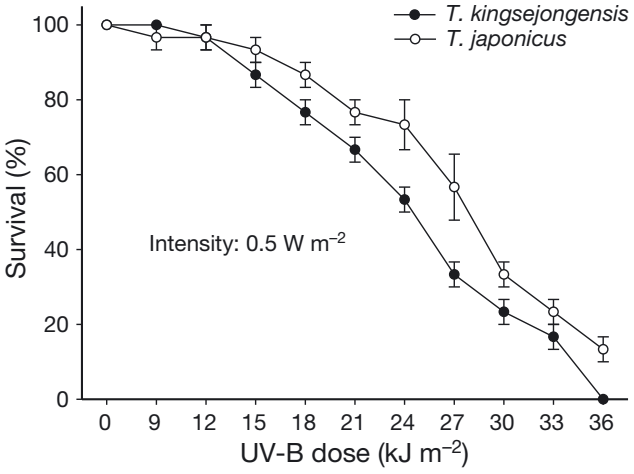


Fig. 2. The survival rate of the copepod *Tigriopus kingsejongensis* compared with *T. japonicus* in response to different doses of UV radiation

Table 1. Genbank accession numbers and primer sets used to measure expression of antioxidant and chaperone genes in copepods *Tigriopus kingsejongensis* and *T. japonicus* in response to UV radiation

| Gene | GenBank acc. no. | Primer sequences (5′–3′) Forwards | Reverse |
|---|---|---|---|
| **Antioxidant genes** | | | |
| *GST-Delta-E* | KX695143 | CAA GGA AAC CTT GGA GAC GTT C | GCC AGT GGC CTC AAT GGA G |
| *GST-Mu3* | KX695144 | GCT GAG TTT GTC CGC TTC CTC | GGG CGT GGCATTGATGATC |
| *GST-Zeta* | KX695145 | CAT GAA GAT GGC ACG CGA TG | CGG CGT TGT AGA TTT GAG GGA C |
| *mGST1* | KX695146 | GAT GCA TCT GAA CGA CAT CGA G | TGT AAG TCA TGG TGT GGC CAA G |
| *mGST3* | KX695147 | AAT TGC GTC CAG CGA GCA C | TCC CAA GCC GTA GAC GAT G |
| *Catalase* | KX695148 | CCC ACG AAC TAC TTT GCC GAG | AAT TGG CCA CTT TGG TCC TG |
| *MnSOD* | KX695149 | AGA CCA TGC AAG AAA GGC TCT C | CAG ACG TCG ATG CCG AAA AG |
| *CuZnSOD* | KX695153 | AAG AAC CAT GGC AAT CCC TTC | AGC TGT ATG AGC TTG TCC ATG |
| *GR* | KX695150 | CAA AAC ACG AGC TCC AAG AAC AC | TCC TGG TCA AAC AAG CGA TGA G |
| *GPx* | KX695151 | GAA CTG CGC AGG AAA TCA AGG | CGT GCT TCA GAT AAG TGA ACA AGG |
| **Chaperone genes** | | | |
| *Hsp10* | KX695152 | CGT TGT TCG ACC GCG TG | AAT GGC GCC CGT ACC CAC |
| *Hsp20* | KX695139 | CAA ATG GAG TCC GAT GCT CAC | TTA TTG TTG GTC GTG ACC TTC AG |
| *Hsp40* | KX695140 | GTG TTC AAA CGC AAC GGC AC | AAC TTC ACC AGG GAT GGT CTG |
| *Hsp70* | KX695141 | TAT CGA AAC TGC CGG TGG TG | AGA TGG TTG TCC TTG GTC ATG G |
| *Hsp90* | KX695142 | CAG CTT TGC CGA CTA CGT GTC | TTC TTG CCG TCG AAC TCC TTG |
| **Housekeeping gene** | | | |
| *18S rRNA* | KX695154 | CAC CGA ACC ACT GGC AAT G | AAA AGT CAG CTC GCA CGG AC |

tion in *T. kingsejongensis* were determined as 23.16 and 12 kJ $m^{-2}$, respectively. In *T. japonicus*, LD50-96h and NOEL were determined as 26.42 and 12 kJ $m^{-2}$, respectively, in response to UV exposure (Fig. 2).

### Measurement of ROS levels and antioxidant enzymatic activities

To examine whether UV radiation induces oxidative stress in *T. kingsejongensis* and *T. japonicus*, the intracellular ROS and activities of the antioxidant enzymes were measured in response to 12 kJ $m^{-2}$ UV exposure (0.5 W $m^{-2}$ intensity) over time (0, 1, 3, and 6 h). ROS levels increased ($p < 0.05$) over time in response to 12 kJ $m^{-2}$ UV exposure, indicating significantly increased ($p < 0.05$) oxidative stress at 3 and 6 h (Fig. 3). Also, the activity of SOD antioxidant enzyme increased in response to UV, but not GR activity (Fig. 3).

### Expression of antioxidant genes in response to UV radiation

To examine the toxic effects of UV radiation at the molecular level, mRNA expression profiles of 10 antioxidant-related genes (*GST-delta-E*, *GST-mu3*, *GST-zeta*, *mGST1*, *mGST3*, *catalase*, *MnSOD*, *CuZnSOD*, *GR*, *GPx*) were measured in *T. kingsejongensis* and *T. japonicus* in response to 12 kJ $m^{-2}$ (0.5 W $m^{-2}$) UV

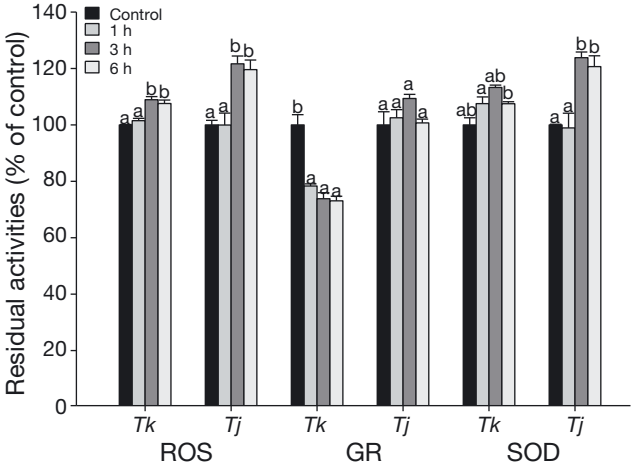


Fig. 3. Response of copepods *Tigriopus kingsejongensis* (*Tk*) and *T. japonicus* (*Tj*) to UV exposure (12 kJ $m^{-2}$, 0.5 W $m^{-2}$): changes over time, at 0 (control), 1, 3, and 6 h, in the generation of intracellular reactive oxygen species (ROS) and activity of the antioxidant-related enzymes glutathione reductase (GR) and superoxide dismutase (SOD). Letters above the columns indicate significant differences based on ANOVA (Tukey's post hoc test, $p < 0.05$)

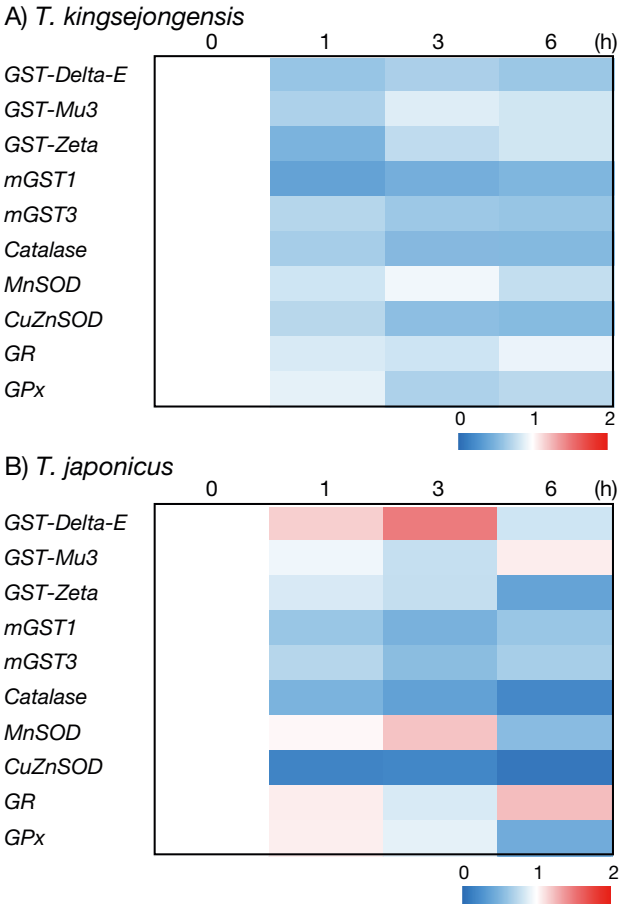


Fig. 4. Heat map showing expression of antioxidant system genes in copepods *Tigriopus kingsejongensis* and *T. japonicus* in response to UV exposure (12 kJ $m^{-2}$, 0.5 W $m^{-2}$) over time, at 0 (control), 1, 3, and 6 h. Blue indicates down-regulation, and red indicates upregulation of mRNA expression compared to control (white)

exposure over time (0, 1, 3, and 6 h). The expression of all antioxidant-related genes decreased significantly in *T. kingsejongensis* ($p < 0.05$) within 6 h in response to 12 kJ $m^{-2}$ UV (Fig. 4A; see also Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m561p099_supp.pdf), while some genes (e.g. *GST-Delta-E*, *MnSOD*, *GR*) showed slight up-regulation in *T. japonicus* (Fig. 4B). This suggests that these 2 congeneric species have a different mode of response to UV radiation.

### Expression of chaperone genes in response to UV radiation

To examine the toxic effects of UV radiation on 2 congeneric *Tigriopus* species, the mRNA expression patterns of 5 *hsp* chaperone genes (*hsp10*, *hsp20*,

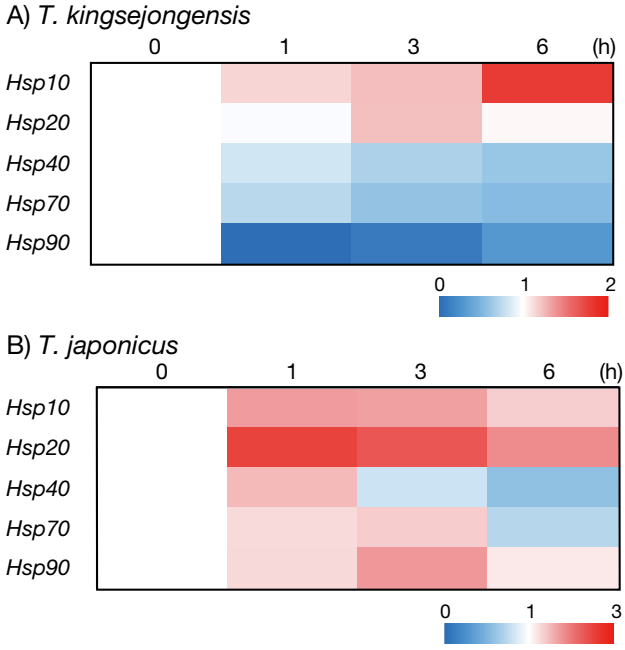


Fig. 5. Heat map showing expression of heat shock protein (*hsp*) chaperone genes in copepods *Tigriopus kingsejongensis* and *T. japonicus* in response to UV exposure (12 kJ $m^{-2}$, 0.5 W $m^{-2}$) over time, at 0 (control), 1, 3, and 6 h. Blue indicates down-regulation, and red indicates up-regulation of mRNA expression compared to control (white)

*hsp40*, *hsp70*, *hsp90*) were measured in *T. kingsejongensis* and *T. japonicus* in response to 12 kJ $m^{-2}$ (0.5 W $m^{-2}$) UV radiation over time (0, 1, 3, and 6 h). Small heat shock protein genes such as *hsp10* and *hsp20* increased significantly ($p < 0.05$) in response to 12 kJ $m^{-2}$ UV radiation. However, other *hsp* genes (*hsp40*, *hsp70*, *hsp90*) decreased significantly ($p < 0.05$) in response to 12 kJ $m^{-2}$ UV in *T. kingsejongensis* (Fig. 5A), but not in *T. japonicus* (Fig. 5B; see also Fig. S2 in the Supplement).

## DISCUSSION

Invertebrates in polar (Antarctic) and temperate regions are exposed to different natural environmental factors due to radiative flux and temperature (Wrona et al. 2006, Young et al. 2006, Häder et al. 2011). Among those, UV radiation has been considered to be a stressor for several physiological processes that cause damage to DNA and inactivate enzymes, leading to detrimental effects on invertebrates (Karanas et al. 1981, Malloy et al. 1997, Wong et al. 2015). In addition, a strong negative impact is known from studies of copepod embryogenesis, feeding, lack of a behavioural response, egg production and survival of nauplii and copepodites (Damkaer & Dey 1982, Lacuna & Uye 2001, Dahms & Lee 2010). The present study contributes to our understanding of UV radiation-induced oxidative stress on different sensitivities of copepods in Antarctic and temperate regions.

In the present study, the LD50-96h and NOEL of UV radiation in ovigerous females of *T. kingsejongensis* were determined as 23.16 and 12 kJ $m^{-2}$, respectively (Fig. 2), while the LD50-96h and NOEL of UV radiation in ovigerous females of *Tigriopus japonicus* were 26.42 and 12.12 kJ $m^{-2}$, respectively (B. M. Kim et al. 2015). Though there is no significant difference in LD50-96h, the observed significant difference in survival in response to UV exposure suggests an adaptive response of these species to UV radiation. To date, acute toxicity values including mortality assessments are important traditional endpoints in examining the physiological activity in toxicological studies in response to environmental stressors (Amiard & Amiard-Triquet 2015). UV radiation is well known to affect survival in diverse marine organisms (Kim et al. 2011, Won et al. 2014, B. M. Kim et al. 2015); however, sensitivities in response to toxicants differ among species, reflected in variations in LD50 values. For example, in the copepod *T. japonicus*, LD50-96h UV is 26.4 kJ $m^{-2}$ (B. M. Kim et al. 2015), while LD50-96h is 4.4 kJ $m^{-2}$ in the copepod *Paracyclopina nana* (Won et al. 2014). This study found that the susceptibility of copepods to UV exposure was significantly different between two congeners despite the lack of significant difference between LD50-96h values. Differences in sensitivity to UV radiation reflect the evolution of antioxidant defense mechanisms associated with enzymatic activities in order to overcome oxidative damage (Pastore et al. 2003, Birben et al. 2012). For example, increased activity of ROS-mediated antioxidant enzymes (e.g. GST, GPx, GR) in response to UV radiation was found in the rotifer *Brachionus koreanus* (Kim et al. 2011) and *T. japonicus* (Kim et al. 2014). An elevated level of GST was also found in the cladoceran crustacean *Daphina magna* (Kim et al. 2009) in response to UV radiation.

To understand how UV radiation leads to the difference between 2 congeneric *Tigriopus* species in survival rate at NOEL values, we measured ROS and antioxidant enzymatic activities of SOD, GST, and GR over time (0, 1, 3, 6 h) in response to UV of 12 kJ $m^{-2}$ with 0.5 W $m^{-2}$ intensity. Intracellular ROS levels were up-regulated to a different extent ($p < 0.05$) between *T. kingsejongensis* and *T. japonicus* (Fig. 3),

suggesting that a possible elevated defense mechanism to oxidative stress is associated with the increased survivability in *T. japonicus* compared to *T. kingsejongensis*. UV radiation-induced oxidative stress and antioxidant defense mechanisms have been identified in many copepods, although enzymatic activities vary in accordance with dose and/or species (Fig. 3). ROS are well known to initiate and catalyze diverse radical reactions in living organisms (Valko et al. 2007) by attacking various biomolecules (e.g. DNA, proteins, and lipids), directly and indirectly, resulting in mutagenesis, cellular ageing, and carcinogenesis (Gniadecki et al. 2001). There have been some studies of ROS production in response to UV exposure in the rotifer *B. koreanus* (Kim et al. 2011) and the copepods *T. japonicus* (B. M. Kim et al. 2015) and *P. nana* (Won et al. 2014). These studies show that ROS generation in response to diverse environmental stressors (e.g. UV radiation, metals, and gamma radiation) is associated with detrimental effects on normal life-cycle parameters (e.g. survival, growth, and reproduction). This suggests that, in our study, UV radiation-induced ROS generation led to harmful effects on normal physiological conditions by inducing DNA damage in *T. kingsejongensis* and *T. japonicus*, even though their underlying molecular responses could be different.

To examine the adaptive defense mechanism of species, expression patterns of antioxidant defense genes (e.g. *GSTs*, *SODs*, *CAT*, *GR, GPx* genes) in *T. kingsejongensis* and *T. japonicus* exposed to 12 kJ $m^{-2}$ UV radiation were measured (Fig. 4). In *T. kingsejongensis,* mRNA expression patterns of all antioxidant-related genes decreased ($p < 0.05$), while in *T. japonicus GST-Delta-E, MnSOD,* and *GR* genes showed up-regulation. These different gene expression patterns in 2 congeneric species indicate the probability of different rates of survival in response to 12 kJ $m^{-2}$ (and/or a higher dose) of UV radiation. A similar difference was observed between gene expression patterns of antioxidant defense genes such as *GSTs*, *SODs*, *CAT*, *GR,* and *GPx* in response to UV exposure in the copepods *T. japonicus* and *P. nana* (B. M. Kim et al. 2015, Won et al. 2014). Antioxidant-related genes have been shown to play important roles in the cellular defense mechanisms of the copepods *T. japonicus* (Lee et al. 2008) and *P. nana* (Won et al. 2014) and the rotifer *B. koreanus* (Han et al. 2016) in response to oxidative damage-induced by marine pollutants.

Exposure to UV radiation can also modulate *hsp* genes, to an extent that depends on the sensitivity of the organism. Previously, *hsp* genes have been shown to play an important role in cellular defense mechanisms in response to environmental stressors (e.g. heat, xenobiotics, UV radiation) (Sarkar 2006). While we observed the modulation of small *hsp* genes in response to UV exposure in both copepods, the temperate copepod *T. japonicus* was more actively responding to UV radiation-induced cellular damage. Small *hsp* genes play an important protective role in response to environmental stressors including UV radiation. For example, expression of *hsp20* was significantly increased by temperature stress and hydrogen peroxide ($H_2O_2$) in the rotifer *B. koreanus* (Rhee et al. 2011). Similarly, in *B. koreanus*, *hsp20* and *hsp27* showed significant up-regulation in response to UV radiation (Kim et al. 2011) and, in the copepod *T. japonicus*, *hsp20* was found to be significantly up-regulated by exposure to heavy metals (e.g. silver, arsenic, copper) and UV radiation (Kim et al. 2014, B. M. Kim et al. 2015), indicating that small *hsp* genes were likely to be involved in repairing cellular damage. Besides, large *hsp* genes (e.g. *hsp70, hsp90*) are considered to be the major cellular proteins protecting cells in response to multiple stressors (Ivanina et al. 2008) by correcting the misfolding of proteins during stress (Kalmar & Greensmith 2009). We have found that *hsp70* and *hsp90* genes in *T. japonicus* were up-regulated to a greater extent than in *T. kingsejongensis*, suggesting that *T. japonicus* could be more tolerant to UV radiation-induced cellular damage. Our observations show that UV radiation could induce oxidative stress that triggered a defense mechanism by modulating the antioxidant enzymes and their related genes. However, the gene expression pattern and enzymatic activity were dissimilar when comparing the 2 climatic regions, suggesting that the Antarctic copepod *T. kingsejongensis* was more susceptible to UV radiation. Earlier studies support this observation, and indicate that natural populations of Antarctic zooplankton are more subject to significant DNA damage due to UV radiation (Malloy et al. 1997, Jarman et al. 1999); even at low irradiation levels, deleterious effects of UV are apparent (Ban et al. 2007) , including reproductive impairment (Karanas et al. 1981). Two other copepod species, *Calanus helgolandicus* and *C.sinicus*, show different responses of stress/detoxification genes between species and among populations when fed on different foods, including the oxylipin-producing diatom *Skeletonema marinoi* and the toxic dinoflagellate *Karenia brevis* (Lauritano et al. 2012a, 2013, 2015, Carotenuto et al. 2014).

In conclusion, the present study reveals that copepods are potentially vulnerable to UV exposure in a

dose-dependent manner. Though there is only a marginal difference in LD50-96h, UV exposure led to different susceptibilities in the survival of the Antarctic copepod *T. kingsejongensis* and the temperate copepod *T. japonicus*, with the latter demonstrating higher survivability. The reduced susceptibility and greater adaptiveness in *T. japonicus* were confirmed at the molecular level, expressed in differences between the antioxidant defense mechanisms of the 2 species. Differences in the modulation of antioxidants and *hsp* gene expressions were likely the reason for better survival in *T. japonicus*. Overall, this study provides a better understanding of how UV radiation affects the survival of 2 congeneric copepod populations in different climatic regions.

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