Aquatic Toxicology 155 (2014) 101–109

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Sublethal gamma irradiation affects reproductive impairment and elevates antioxidant enzyme and DNA repair activities in the monogonont rotifer *Brachionus koreanus*

Jeonghoon Han[a,1], Eun-Ji Won[a,1], Il-Chan Kim[b], Joung Han Yim[b], Su-Jae Lee[c], Jae-Seong Lee[a,*]

[a] Department of Biological Sciences, College of Science, Sungkyunkwan University, Suwon 440-746, South Korea
[b] Division of Life Sciences, Korea Polar Research Institute, Incheon 406-840, South Korea
[c] Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, South Korea

ARTICLE INFO

Article history:
Received 31 March 2014
Received in revised form 13 June 2014
Accepted 17 June 2014
Available online 21 June 2014

Keywords:
Gamma radiation
Offspring
Reactive oxygen species
DNA damage
DNA repair
Rotifer

ABSTRACT

To examine the effects of gamma radiation on marine organisms, we irradiated several doses of gamma ray to the microzooplankton *Brachionus koreanus*, and measured in vivo and in vitro endpoints including the survival rate, lifespan, fecundity, population growth, gamma ray-induced oxidative stress, and modulated patterns of enzyme activities and gene expressions after DNA damage. After gamma radiation, no individuals showed any mortality within 96 h even at a high intensity (1200 Gy). However, a reduced fecundity (e.g. cumulated number of offspring) of *B. koreanus* at over 150 Gy was observed along with a slight decrease in lifespan. At 150 Gy and 200 Gy, the reduced fecundity of the rotifers led to a significant decrease in population growth, although in the second generation the population growth pattern was not affected even at 200 Gy when compared to the control group. At sub-lethal doses, reactive oxygen species (ROS) levels dose-dependently increased with GST enzyme activity. In addition, up-regulations of the antioxidant and chaperoning genes in response to gamma radiation were able to recover cellular damages, and life table parameters were significantly influenced, particularly with regard to fecundity. DNA repair-associated genes showed significantly up-regulated expression patterns in response to sub-lethal doses (150 and 200 Gy), as shown in the expression of the gamma-irradiated *B. koreanus p53* gene, suggesting that these sublethal doses were not significantly fatal to *B. koreanus* but induced DNA damages leading to a decrease of the population size.

1. Introduction

The ionizing radiation (IR), generated by the decaying processes of radionuclides, induces adverse effects on organisms from the DNA to the individual level (e.g. DNA, chromosomal aberrations, cell, protein, growth, reproduction, community, etc.) (Fischbein et al., 1997; Kovalchuk et al., 1999; Zaka et al., 2004; Fuma et al., 2010; Kang et al., 2012; Rhee et al., 2012). Among the various residual effects on organisms, oxidative stress and single/double strand breaks (SSBs, DSBs) of DNA are the most common and severe symptoms observed in gamma irradiated organisms (Rhee et al., 2012; Won and Lee, 2014). Gamma-induced reactive oxygen species (ROS) cause severe damage to cellular macromolecules, including DNA (Barzilai and Yamamoto, 2004). After the Chernobyl nuclear power plant accident, IR effects were considered as a matter of great concern. For example, a high frequency of germline mutations was observed in children (Dubrova et al., 1996). Furthermore, the salvage workers from the Chernobyl nuclear power plant accident suffered from amorphous sperm heads, affecting their fertility (Fischbein et al., 1997), suggesting that IR induces significant repercussions on the individual level.

The Fukushima nuclear power plant incident in 2011 also brought to light the ecological and health risks associated with nuclear accidents, as huge amounts of nuclides spilled directly into the Pacific Ocean (Kameník et al., 2013; Kumamoto et al., 2013). In fact, high activity concentrations of ^{134}Cs in zooplankton were observed in the northwest Pacific Ocean 10 months after the damage to the Fukushima Dai-ichi nuclear power plant (Kitamura et al., 2013). As these nuclides are released into the surrounding areas

* Corresponding author. Tel.: +82 31 290 7011.
E-mail address: jslee2@skku.edu (J.-S. Lee).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aquatox.2014.06.009
0166-445X/

via sea currents, they emit radiation directly that accumulates in marine organisms. Furthermore, this has worldwide consequences on humans, as people can be exposed by consuming seafood (Fisher et al., 2013). However, limited information concerning the repercussions of IR in aquatic ecosystems was available, particularly in terms of invertebrates, as many previous studies have focused on diverse biota in the terrestrial environment and only some on aquatic vertebrates (Mitani and Egami, 1982; Krivolutzkii and Pokarzhevskii, 1992; Garnier-Laplace et al., 2011; Rhee et al., 2012). In fish, IR has been shown to increase expressions of DNA repair related genes and induce DNA impairment at the molecular level (Rhee et al., 2012).

Of the marine invertebrates, micro-zooplankton plays a pivotal role in the marine food web and is suitable for ecophysiological studies. Among the micro-zooplankton, rotifers have been considered a good model for studying life table parameters for a lifespan or even several generations, as they have a short lifespan (~24 h) (Bozovic and Enesco, 1986; Austad, 2009; Dahms et al., 2011). Also, the rotifer *Brachionus koreanus* has good characteristics for ecotoxicological studies due to its: small size (≈150 μm), small genome size (≈120 Mb), and the ability to perform well at laboratory conditions. Additionally, massive RNA-seq (mRNA no. 61,005; assembled mRNA length 87,724,829 bp; N50 3251 bp) and genomic DNA (Scaffold no. 106,150; assembled scaffold length 119,594,691 bp; N50 1747 bp; average 1127 bp) databases are available from the rotifer *B. koreanus*, allowing this species to act as a good non-model organism to uncover the modulatory effect of diverse defense genes (e.g. antioxidant, DNA repair, molecular chaperoning) in response to IR exposure. Molecular information on molecular defensomes (e.g. oxidative stress related genes and others) and diverse DNA repair machineries such as *DNA-PK*, *Ku70*, *Ku80*, nucleotide excision repair (NER), homologous recombination (HR), mismatch repair (MMR), single-strand DNA binding protein (SSB) and others in several environmental stress conditions (e.g. hydrogen peroxide, UV-B radiation, and heavy metal exposure) are available in *B. koreanus* (Kim et al., 2011; Jung and Lee, 2012; Han et al., 2013), providing that the elucidated mechanistic approach is possible in response to the gamma radiation in the rotifer. However, most studies have focused on the molecular mechanisms at the levels of transcriptomes and enzyme activities, linking molecular events with ecological relevance.

In this paper, we measured the survival rate, population growth, and reproduction rates as endpoints of *B. koreanus* life parameters for different doses of gamma radiation to fill out the knowledge gap between in vivo and in vitro studies on the impairment of reproduction and DNA damages in response to gamma radiation. We also analyzed intracellular oxidative stress induced by IR, along with modulations of gene expressions to provide a better understanding of oxidative stress and DNA damage upon gamma radiation.

2. Materials and methods

2.1. Culture of the rotifer B. koreanus

Individual *B. koreanus* samples were isolated and reared in artificial seawater adjusted to 15 psu salinity and a temperature of 25 °C under a LD 12:12 h photoperiod. They were fed a diet of the green algae *Tetraselmis suecica*. The *B. koreanus* used in this study reproduces only by parthenogenesis. Species identification was confirmed through their morphological characteristics (Lee et al., 2011), using the sequence analysis of the mitochondrial DNA cytochrome oxidase I (*CO1*) as the barcoding gene (Hwang et al., 2013).

2.2. Gamma radiation and its effects on mortality, lifespan, and fecundity

For gamma irradiation, Gammacell® 1000 Elite (a radiation intensity, 2 Gy/min; MDS Inc., Ottawa, ON, Canada) was used at 25 °C. To examine the effects of gamma radiation on mortality, 10 individuals were irradiated to 200, 400, 600, 800, 1000, and 1200 Gy, and an un-irradiated group was used as a control. Mortality was measured by counting the number of individuals who survived 96 h after gamma radiation. To measure the effect of gamma irradiation on life span and fecundity for the rotifer *B. koreanus*, we exposed neonates to diverse ranges of gamma radiation (0, 50, 100, 150, and 200 Gy). To obtain just-hatched individuals, we isolated eggs from ovigerous females, as described in a previous study (Kim et al., 2011). Eight newborn neonates (age <2 h) were collected and were exposed to each of the aforementioned doses of gamma radiation. The average lifespan and the number of cumulated offspring were counted until their death.

2.3. Effects of gamma radiation on population growth retardation

To measure the growth retardation after gamma radiation, we counted the number of rotifers over a 10 day period following their exposure to gamma radiation (0, 50, 100, 150, and 200 Gy). Furthermore, 10 neonates were isolated from 0 Gy- to 200 Gy-irradiated groups and the population growth rate of the second generation (w/o gamma radiation) was measured under the same conditions. During the experiment, we supplied algal diets of *T. suecica* (approximately 5×10^4 cells/ml) every 24 h.

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

To analyze the gamma radiation-induced oxidative stress levels, *B. koreanus* was exposed to various levels of gamma radiation (0, 50, 100, 150, and 200 Gy). Intracellular ROS were measured using the dichlorofluorescin diacetate (DCFDA) fluorescence method (Kim et al., 2011) at 1 hr after gamma irradiation. Samples were briefly 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 in a 13,200 × *g* for 15 min (4 °C). The supernatants reacted with H_2DCFDA and their wavelengths were measured at 485 nm for excitation and 520 nm for emission (Thermo Scientific Co., Varioscan Flash, Vantaa, Finland).

The activity of the GST (EC 2.5.1.18) was measured using a GST assay kit (Sigma–Aldrich Co., St. Louis, MO, USA). The activities of the GST were calculated according to the reduced absorbance with 1-chloro-2,4-dinitrobenzene (CDNB) using a spectrophotometer (Ultrospec 2100 Pro, Amersham Bioscience, Cambridge, UK). The total protein contents were determined in order to normalize the ROS contents and GST activities, in a dye binding method using a bovine serum albumin as its standard (Bradford, 1976).

2.5. Messenger RNA expressions of p53, DNA repair-related, and antioxidant genes

To measure the expression patterns of target genes, we irradiated sublethal doses (150 and 200 Gy) to *B. koreanus* and measured the mRNA expression 0, 20, 40, 60, 180, and 360 min after gamma radiation. The total RNAs were extracted from the irradiated-*B. koreanus* with TRIZOL® reagent (Invitrogen, Paisley, Scotland, UK) according to the manufacturer's instructions. The quantity and quality of the total RNA was checked at 230, 260, and 280 nm using a spectrophotometer (Ultrospec 2100 Pro). To synthesize the cDNA for real-time RT-PCR (real-time RT-PCR), 2 μg of the total

RNA and oligo(dT)$_{20}$ primer were used for reverse transcription (SuperScript™ III RT kit, Invitrogen, Carlsbad, CA, USA).

Real-time RT-PCR was conducted for the following conditions: 95 °C/4 min; 35 cycles of 95 °C/30 s, 58 °C/30 s, 72 °C/30 s; 72 °C/10 min using SYBR Green as a probe (Molecular Probe, Invitrogen). To confirm the amplification of specific products, melting curve cycles were run for the following conditions: 95 °C/1 min, 55 °C/1 min, 80 cycles of 55 °C/10 s, with a 0.5 °C increase per cycle using real-time RT-PCR F or R primers (Suppl. Table 1). All of the experiments were performed in triplicate. Fold change, which we used to compare the relative gene expression to the controls, was determined using the $2^{-\Delta\Delta C}$t method (Livak and Schmittgen, 2001).

2.6. Statistical analysis

All of the results were expressed as a mean value. The normal distribution and homogeneity of variances of data were checked using Levene's test. Data were analyzed using one-way ANOVA, followed by Tukey's honesty significant difference test and *t*-test ($P < 0.05$). All the statistical analyses were performed using SPSS® version 21 software (SPSS Inc., Chicago, IL, USA)

3. Results

3.1. Mortality, life span, and cumulative offspring

The lethal dose, however, was not measured until 1200 Gy at 96 h after radiation (Suppl. Fig. 1). The lifespan of a gamma irradiated rotifer was reduced when doses were increased; however, statistical significances were not observed between the different groups (Fig. 1A). The offspring, however, was significantly reduced to 20% and 6% in 150 and 200 Gy-irradiated groups compared to the control, respectively (Fig. 1B).

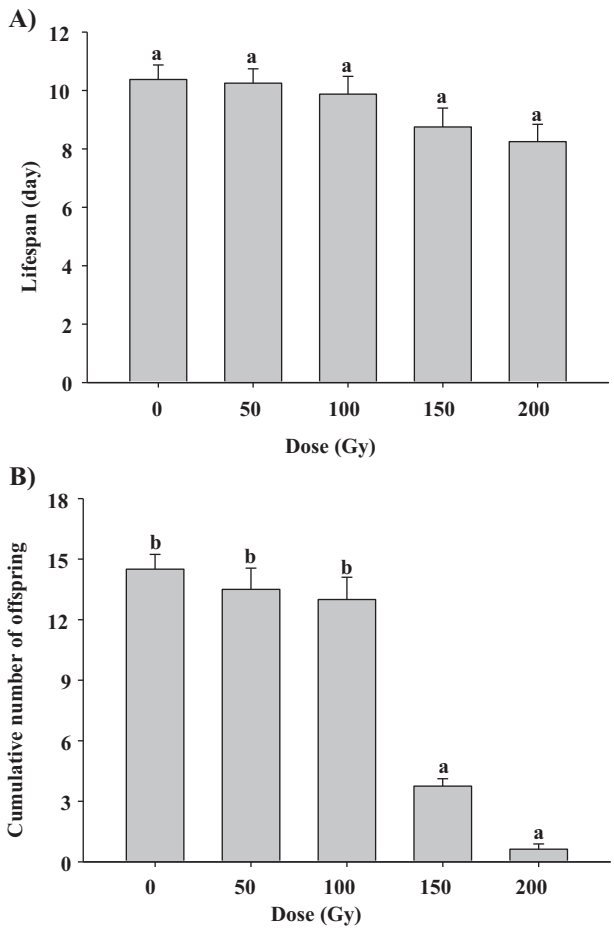

Fig. 1. Damages of gamma irradiation on life table parameters: (A) lifespan (day) and (B) the cumulative number of offspring from ovigerous females. Significant differences were analyzed by ANOVA (Tukey's post hoc test; $P < 0.05$) and expressed as different letters.

3.2. Population growth retardation

Compared to the control group, 150 and 200 Gy-irradiated rotifers showed growth retardation during the lag phase (Fig. 2A, $P < 0.05$). However, log–log plots from their exponential growth phases show similar regression coefficients (regression coefficient = 5.5–6.2; Fig. 2B) between the differently irradiated groups. Interestingly, the sigmoidal growth curve of 200 Gy-irradiated second generations (w/o gamma radiation) had the same trend as that of the control (Fig. 2C).

3.3. Production of ROS and the activity of GST

Oxidative stress status under gamma radiation was measured in *B. koreanus*. After gamma radiation, all of the groups generated ROS significantly ($P < 0.05$; Fig. 3A), showing the highest level at 150 Gy. Antioxidant enzyme GST activity also significantly increased at 100 and 150 Gy (Fig. 3B).

3.4. Messenger RNA expressions of antioxidant related, heat shock protein, and DNA repair related genes

Expression patterns of antioxidant, chaperoning, and DNA repair related genes were elevated in response to gamma radiation (Fig. 4 and Suppl. Fig. 3). For the *p53* gene, *B. koreanus* showed a bell-shaped response over time depending on the irradiated doses (Fig. 5). Also, the 200 Gy gamma-irradiated *B. koreanus* group showed a more elevated expression than the 150 Gy-irradiated group, indicating that the 200 Gy-irradiated group was more significantly damaged than the 150 Gy-irradiated group. Eighteen DNA repair-related genes had modulated mRNA expressions upon gamma-irradiation, and the degree of the expression levels was different according to the function of the DNA repair mechanisms (Fig. 6 and Suppl. Fig. 4).

4. Discussion

Nuclear contamination is not limited to the local environment, as shown in the case of the Fukushima nuclear power plant incident. However, the risks of radiation on aquatic vertebrates and invertebrates, such as fish and microbial communities, have not been adequately studied as yet (Fuma et al., 2010; Rhee et al., 2012). The effects of radionuclides on marine zooplankton are emerging concerns, as marine plankton plays a pivotal role in connecting autotrophs and large heterotrophs in the marine food web and the global carbon and nutrient cycles (Keister and Bonnet, 2012). Zooplankton plays an important role in concentrating and transporting natural radionuclides in the Mediterranean Sea (Krishnaswami et al., 1985). For example, in brine shrimp, the concentrations of natural-occurring nuclides ^{210}Po and ^{210}Pb can increase by 5–12 times more than those in phytoplankton through food web bioaccumulation (Stewart et al., 2005). Thus, studying gamma radiation-induced effects and repair systems using zooplankton that connects within food webs can improve our understandings of the implications of IR in the marine environment. This type of study can estimate how mechanistic findings in zooplankton with in vivo endpoints can be linked to phenomena within whole ecosystems.

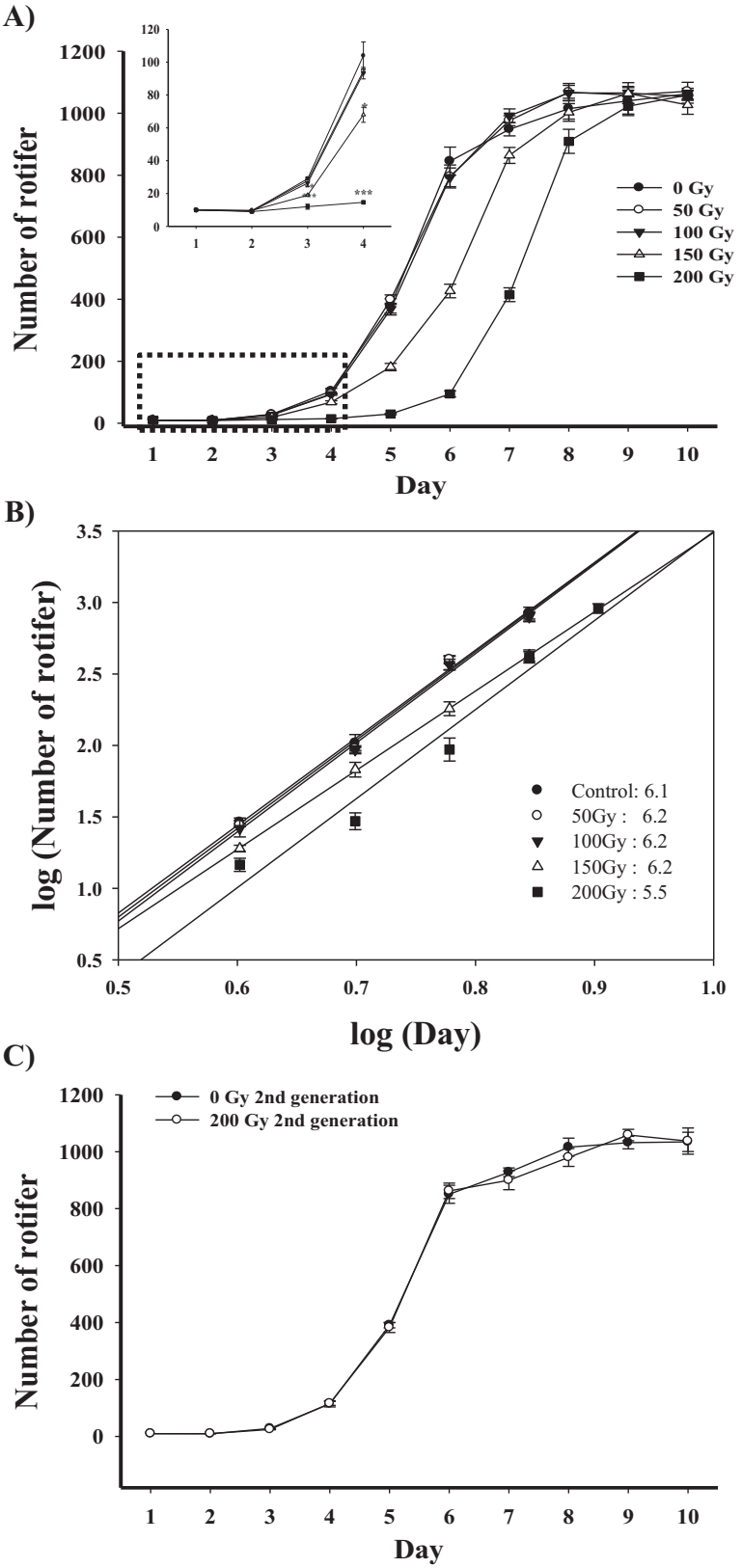

Fig. 2. Effect of gamma radiation on fecundity level: (A) the population growth rate upon gamma irradiation (0, 50, 100, 150, and 200 Gy) for 10 days, (B) log–log plots from the exponential growth phase of each group, and (C) the population growth observed in second generations from control (0 Gy) and gamma irradiated individuals (200 Gy) for 10 days. Error bars indicate mean ± SE.

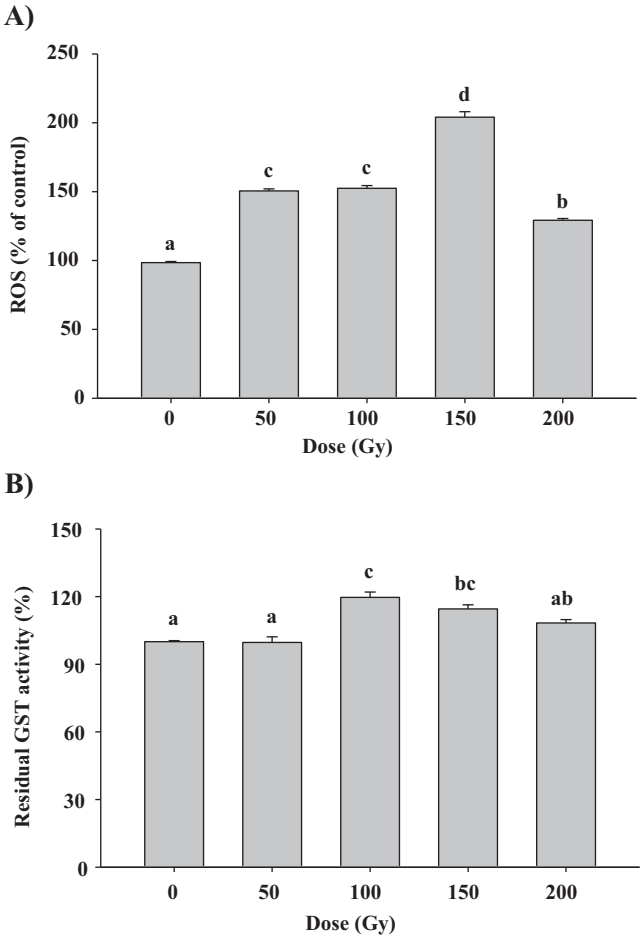

Fig. 3. Effects of gamma irradiation (0, 50, 100, 150, and 200 Gy) on the generation of intracellular reactive oxygen species (ROS) and enzyme activity of glutathione *S*-transferase (GST). Significant differences were analyzed by ANOVA (Tukey's post hoc test; $P < 0.05$) and expressed as different letters.

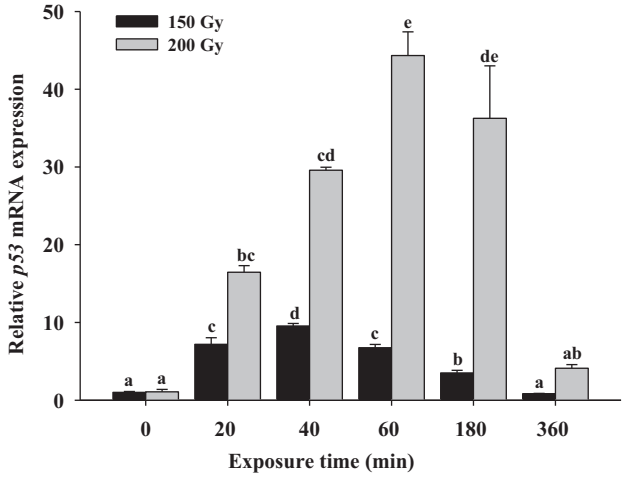

Fig. 5. Effects of gamma irradiation (150 and 200 Gy) for time-courses (0, 20, 40, 60, 180, and 360 min) on *p53* gene. Values are the means of three replicate samples and data are shown as mean ± SD. Different letters indicate significant differences observed in each dose (Tukey's post hoc test; $P < 0.05$).

The monogonont rotifer *B. koreanus* shows great tolerance to gamma radiation. In terms of the susceptibility of vertebrates and invertebrates to IR, larger herbivorous mammals are most vulnerable to IR, followed by smaller mammals, insects, aquatic invertebrates, and plants (Driver, 1994), suggesting that invertebrates are more tolerant to gamma radiation than vertebrates. In most mammals, less than 2 Gy of gamma radiation induces acute toxicity (Myers, 1989). However, in microcosm experiments microbial communities were not significantly affected, even at 100 Gy (Fuma et al., 2010). The intertidal copepod *Tigriopus japonicus* showed no mortality, even at 7 days with 400 Gy of gamma radiation (Han et al., 2014). It has been reported, actually, that the haploid form, which lacks homologous recombination, is more sensitive for use as a template for repairing IR-induced double strand breaks (DSBs); on the other hand, diploid cells are more resistant because recombinational repair can occur throughout the cell cycle using homologous chromosomes (Westmoreland et al., 2004). Thus, the susceptibility of organisms to IR exposure is closely related to both control of the repair processes and the regulation of the cell cycle kinetics (Cassidy et al., 2007). Rotifers are multicellular and post-mitotic organisms which reproduce by diploid parthenogenesis. In the case of the rotifer *Brachionus*, they are diploid and produce parthenogenetically diploid eggs during mitotic events that are genetically identical to parental individuals.

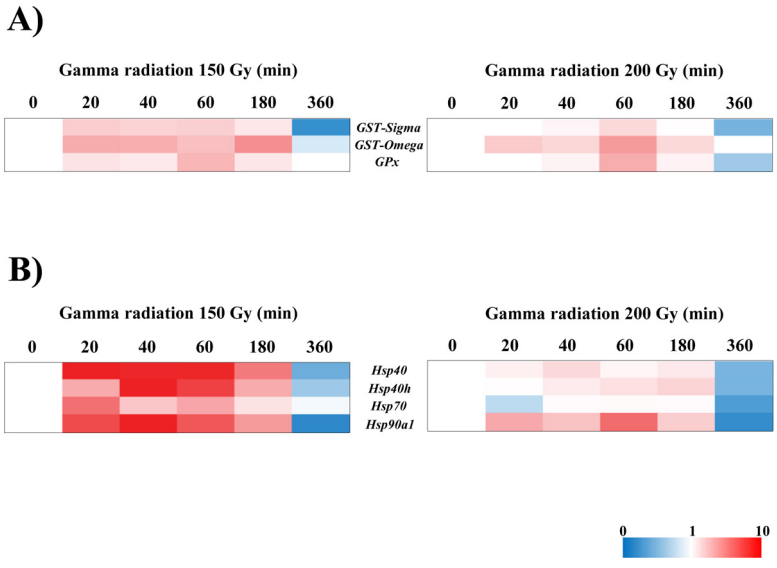

Fig. 4. Effects of gamma irradiation (150 and 200 Gy) for time-courses (0, 20, 40, 60, 180, and 360 min) on (A) glutathione related enzyme (*BK-GSTs* and *BK-GPx*) and (B) *Hsps*.

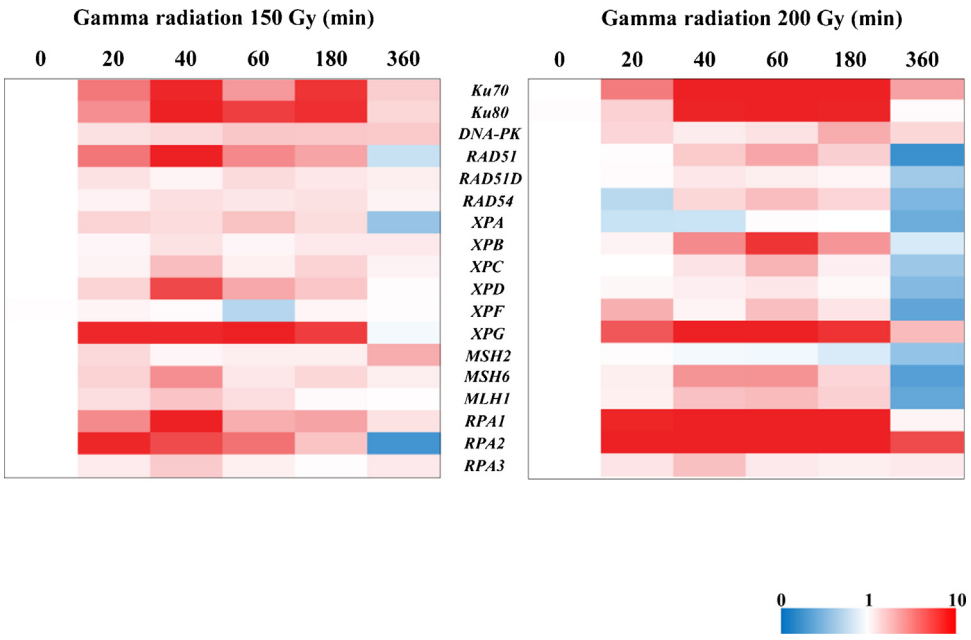

Fig. 6. Effects of gamma irradiation (150 and 200 Gy) for time-courses (0, 20, 40, 60, 180, and 360 min) on DNA repair related genes.

Therefore, rotifers have a high resistance to IR compared to organisms using haploid germ cells for reproduction through active DNA repair systems using homologous chromosome templates. In previous studies, the bdelloid rotifer *Adineta vaga* showed also great tolerance to IR with an unusually effective system of antioxidant protection (Krisko et al., 2012).

The life span, reproductive success, and population growth are reliable indices to examine the implication of gamma radiation, as these parameters are closely associated with the fluctuation of population structures in ecosystem. Rotifer lifespans are slightly reduced in response to gamma radiation although there was a tendency toward decreased lifespan with high levels of irradiated doses. However, this reduction was not statistically significant (Fig. 1A). In the worm *Caenorhabditis elegans*, a reduced lifespan was also observed in response to gamma radiation above 100 krad (Johnson and Hartman, 1988), while an unirradiated radiation-sensitive mutant group showed a lifespan similar to the wild-type. In the rotifer *Asplanchna brighwelli*, degenerative aging was induced by free radical reactions (Bozovic and Enesco, 1986). Thus, IR-induced oxidative stress can affect the lifespan of rotifers in response to gamma radiation. In terms of fecundity, the number of offspring was significantly reduced in response to gamma radiation (Fig. 1B). Fecundity patterns of rotifers were highly associated with lifespan. For example, individuals with long lifespans showed a low fecundity but the opposite was found for short lifespan rotifers in their age classes due to tradeoff mechanisms (Jennings and Lynch, 1928; Snell and King, 1977). Thus, rates of reduced cumulative offspring, seen at levels exceeding 150 Gy, imply that gamma radiation is detrimental to fecundity, as it causes them to allocate their energy to the recovery of oxidative stress. Similarly, in mussels *Mytilus galloprovincialis* and *Perna canaliculus*, environmental stress has been linked to a reduced energy allocation toward reproduction, causing impaired growth (Petes et al., 2007). In addition, in vertebrates, reduced breeding has been caused by a reduction of the testis mass and sperm contents, and a depletion of spermatogonia in response to gamma radiation, as shown in the following fish species: plaice *Pleuronectes platessa*, eelpout *Zoarces viviparus*, guppy *Poecilia reticulata*, and rainbow trout *Onchorhynchus mykiss* (Woodhead, 1977; Knowles, 1992, 1999; Greenwood and Knowles, 1995).

Generally, the inhibition of population growth is one of the potential indices for environmental conditions and the resulting physiological status, reflecting relative abundances in ecosystems, particularly in asexual reproductive organisms such as phytoplankton and rotifers (Yoshinaga et al., 2001; Haney and Jackson, 1996; Boëchat and Adrian, 2006). In the rotifer *Brachionus plicatilis*, birth rate was the key density-dependent factor for regulating population growth in laboratory rotifer populations (Yoshinaga et al., 2001). An excess of 150 Gy in *B. koreanus* caused right-shifted sigmoidal patterns of the population growth curve (Fig. 2A). Under lab conditions, the *Brachionus* sp. population growth has four phases (Yúfera and Navarro, 1995): lag, exponential, post-exponential growth, and declining and/or stationary phase. After gamma radiation, a growth retardation in the lag phase was observed, reaching a stationary phases after a two-day delay, suggesting that gamma radiation leads to only temporary reproductive impairment in *B. koreanus*. However, in all the groups, similar slopes of the exponential phases were observed with statistical significance (Fig. 2B). Furthermore, the same patterns of sigmoidal growth were observed in the second generation between the control and the 200 Gy-irradiated group (both unirradiated groups at the second generation) (Fig. 2C). Similarly, in rotifer *B. plicatilis*, a population development delay of one or two days in the lag phase was observed in groups with copper accumulated diets (Moreno-Garrido et al., 1999). In the water flea *Daphnia magna*, a decrease of metabolic cost for growth and reproduction was also observed as controlling body maintenance and increasing survival in response to alpha radiation (Alonzo et al., 2006). This suggests, therefore, that the trade-off mechanism resides between repair and reproduction under gamma irradiation and that *B. koreanus* has the ability to recover gamma radiation-induced damages on fecundity in two generations.

Gamma radiation can generate ROS directly by interacting with the water in the cells of organisms (Kang et al., 2012). Organisms have evolved to control radicals using antioxidant defenses and enzyme systems in response to IR-induced oxidative stress, leading to diminished ROS (Riley, 1994). In the intertidal copepod *T. japonicus*, gamma radiation induces ROS and leads to a recruiting of DNA repair mechanisms for several antioxidant enzymes, such as GST, glutathione reductase, and GPx, to diminish oxidative

stress (Han et al., 2014). Likewise, *B. koreanus* has a fundamental defense mechanism which counters gamma radiation-induced oxidative stress (Fig. 3). Additionally, the upregulated expression patterns of glutathione-related genes, such as *GST*s (*BK-GST-sigma*, *BK-GST-omega*, and *BK-GST-zeta*) and *BK-GPx*, were also observed in response to gamma radiation (Fig. 4A, Suppl. Figs. 2 and 3(A)), suggesting that glutathione-related antioxidant mechanisms are actively involved in recovering oxidative stress-induced damages in gamma-irradiated *B. koreanus*. In the marine copepod *Paracyclopina nana*, gamma radiation-induced ROS has been associated with retarded growth rates, along with impaired fertility (Won and Lee, 2014). Additionally, the amount of ROS determines sperm quality and correlates to the amount of live and non-apoptotic status, as well as reproduction rates (Marques et al., 2014). Thus, ROS generated by other environmental stresses (e.g. UV-B, IR, temperature, pollutants, etc.) is a triggering factor on life cycle stages, such as hatching, growth, and reproduction, in phytoplankton, zooplankton, and bivalves (Petes et al., 2007; Fuma et al., 2010; Kim et al., 2011; Han et al., 2014).

The relative mRNA expressions of *Hsp* genes were increased in gamma irradiated *B. koreanus* (Fig. 4B, Suppl. Fig. 2 and Suppl. Fig. 3(B)). *Hsps* play a prominent role in protein homeostasis by regulating the protein folding quality control (Nollen and Morimoto, 2002; Imai et al., 2003), and has also been considered to be a stress-induced protein, preventing irreversible aggregation with other proteins in the cells by translocating or refolding stress-induced denatured protein (Nollen and Morimoto, 2002). The primary cellular defense mechanisms of Hsp proteins in response to proteome damage are the chaperoning of the denatured proteins and degradation of the damaged proteins (Parsells and Lindquist, 1993; Nollen and Morimoto, 2002). The released Hsp70 protein binds to aberrant polypeptides/proteins to rescue them, through folding and unfolding with other proteins, to protect the cell from the destructive effects of gamma radiation in human lymphocytes (Parsells and Lindquist, 1993). Furthermore, the involvement of Hsp proteins in response to IR was reported in radiation-induced fibrosarcoma cells and radiation-induced cell death (Park et al., 2000; Lee et al., 2001). Radiation-induced fibrosarcoma cells obtained the ability to adaptively respond to the conditions of inducible Hsp70-transfected cells (Park et al., 2000), while Hsp70-silenced cells showed significant increases of cell killing compared to the control (Du et al., 2009). This implies that an increase in *Hsp* genes, including *Hsp30*, *Hsp40*, *Hsp70*, and *Hsp90*, can be related to the recovery of IR-induced oxidative stress, as seen in *B. koreanus*. However, reduced ROS levels and antioxidant enzymes including glutathione related genes and *hsp* observed at 200 Gy can be caused by overstretched damages in response to IR and/or be caused by reallocation of the energy budget from antioxidant to the DNA repair first.

The *p*53 gene was significantly expressed in a time course manner in response to gamma radiation in *B. koreanus* (Fig. 5). In *p*53-deficient mice, tumor latency was significantly decreased (Kemp et al., 1994), indicating that the *p*53 tumor suppressor gene is a key player in examining DNA damage and protecting the genome in view of cell cycle regulation and apoptosis (Backlund et al., 2001). In the hermaphroditic fish *K. marmoratus* larvae, the *p*53 gene was up-regulated with a sublethal dose of gamma radiation, suggesting that IR can activate the DNA repair system through the involvement of p53 (Rhee et al., 2013). Other key genes, such as DNA repair-related genes (e.g. *Ku70*, *Ku80*, *DNA-PK*) and the *PCNA* gene, were significantly expressed by gamma radiation, seen in their increased susceptibility of cellular damage (Han et al., 2014). Thus, an elevated expression of *B. koreanus p53* is closely linked with cellular defenses to IR as a marker for radiation-induced alterations in gamma irradiated *B. koreanus*.

In vivo and in vitro parameters for gamma radiation damage are closely associated with gamma ray-induced DNA damages. IR generates DNA damages, such as single- and double-strand DNA breaks, basic sites, and alterations of DNA bases (Ward and Kuo, 1976; Rhee et al., 2013). However, in diverse aquatic organisms, repair mechanisms have been reported as recovering DNA damages in response to gamma irradiation through the expression of DNA repair-related genes by detecting genome impairment (Rhee et al., 2013; Han et al., 2014). For example, after exposure to X-ray and gamma radiation, in fathead minnow *Pimephales promelas*, DNA strand breaks disappeared through a linear rate of DNA repair. In addition, the molecular mode of the action of defense mechanisms in response to gamma radiation was observed in the mangrove killifish *K. marmoratus* and copepod *T. japonicus* (Rhee et al., 2013; Han et al., 2014). In this study, the expression patterns of 18 different DNA repair associated genes were examined early in the event of DNA repair in a time-course manner in 150 and 200 Gy gamma-irradiated *B. koreanus* (Fig. 6 and Suppl. Fig. 4). Non-homologous end joining (NHEJ) was considered to be the most important repair mechanism for forming the heterotrimer of *Ku70* and *Ku80* with *DNA-PK* in response to DSBs (Rhee et al., 2012). In general, Ku70/Ku80 heterodimers recruit DNA-PK to form the core protein complex, thus promoting a synapsis of the broken DNA ends in DSBs (Spagnolo et al., 2006). In *B. koreanus*, significant expression patterns of *Ku70*, *Ku 80*, and *DNA-PK* were observed as being key components of NHEJ according to time- and dose-dependent manners (Fig. 6 and Suppl. Fig. 4(A)). HR, required for DSBs repair systems following IR exposure, was also induced in response to gamma radiation (Groth et al., 2012). HR is predominantly induced cell cycle-dependently in S and G2 phases, while NHEJ contributes to the DSBs throughout the cell cycle (Hartlerode et al., 2011). In *B. koreanus*, *RAD* (*RAD51*, *RAD51D*, and *RAD54*) genes were up-regulated in gamma-irradiated *B. koreanus* up to 10 times more than the control group (Fig. 6 and Suppl. Fig. 4(B)). RAD51 plays a pivotal role in initiating HR pathways by assembling a single-stranded DNA to form a helical nucleoprotein that promotes DNA strand exchange (Mazin et al., 2003). Subsequently, RAD54 regulates the stability of RAD51 and stimulates the exchange of DNA strands between the homologous damaged and undamaged DNA molecules (Mazin et al., 2003). In fact, in mammalian cells, the overexpression of RAD51 proteins stimulates HR pathways and increases resistance to IR (Vispé et al., 1998). In a homozygous *RAD54* mutant mouse (*RAD54* −/−), the HR response was significantly reduced in response to IR (Essers et al., 1997). Taken together, these major pathways (e.g. NHEJ and HR) either directly or indirectly regulate the DSB's repair mechanism in *B. koreanus*.

IR also induces single-strand DNA breaks (SSBs) at the base and DNA backbone levels, which are using different repair mechanisms such as nucleotide excision repair (NER) and mismatch repair (MMR). Of the SSB's repair mechanisms, replication protein A (RPA) is responsible for base excision repair. The RPA and *Xeroderma pigmentosum* variant (XPV; e.g. XPA-RPA) protein complex is involved in the initial detection of DNA damages on the SSBs in mammalian DNA (Kobayashi et al., 1998), as for gamma radiation-induced phosphorylation of RPA in human cells (Liu and Weaver, 1993). Also, a germ line of XPC-knockout mice showed significantly higher radiation-induced mutation rates than that in wild-type mice (Miccoli et al., 2007). Thus, the elevated expression of *B. koreanus XPV* (*XPA-G*), as a marker encoding the recognition protein in order to find a DNA adduct, showed that the NER mechanism is likely to have been affected by sublethal doses of gamma radiation in *B. koreanus* (Fig. 6 and Suppl. Fig. 4(C)). Also, MSH2, MSH6, and MLH proteins were required to repair mismatches during DNA replication (Drotschmann et al., 2005). In this study, a mismatch repair (MMR) system as a post-replicative repair of mismatched SSDs, was up-regulated depending on the dose- and time course

manner (Fig. 6 and Suppl. Fig. 4(D)). MSH6 is a significant factor in MMR pathways, as a key component of the MSH2/MSH6 complex, and *MSH6*-deficient cells were hypersensitive to IR-induced cell death and were associated with DSB repair by inducing Ku70 in human cell lines (Shahi et al., 2011). Moreover, *Bk-RPA1*, *Bk-RPA2*, and *Bk-RPA3* genes were significantly induced, particularly in 200 Gy, up to 60 times more than those in the control group (Fig. 6 and Suppl. Fig. 4(E)). RPA is involved in multiple stages of DNA MMR pathways, and RPA plays a role, not only in recruiting MMR initiation factors to the excision initiation point, but in protecting template DNA from the attack of nucleases as essential components of the MMR system (Guo et al., 2006). Thus, in *B. koreanus*, NER, MMR, and SSB-involved damage repairing systems responded effectively to exposure to gamma radiation, initiating a recovery of the cellular damages.

In summary, *B. koreanus* recovers oxidative stress-induced cellular and DNA damage caused by gamma radiation through subsequent defense mechanisms, such as: antioxidants, chaperoning processes, and DNA repair pathways. In this paper, we suggest a causal correlation between in vivo parameters (e.g. survival rate, life span, fecundity, growth retardation) and several molecular indices (e.g. defense mechanism) in *B. koreanus* after gamma radiation exposure. This paper provides a better understanding of how gamma radiation affects individuals and population structure levels as well as molecular levels.

Acknowledgements

We thank Prof. Hans-U. Dahms for his comments on the manuscript, and also thank two anonymous reviewers for their valuable comments to improve the revised manuscript. This work was supported by a grant for the project “Korea-Polar Ocean Development: K-POD” (project no. PM13030) funded by the Ministry of Oceans and Fisheries, Korea.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquatox.2014.06.009.

References

Alonzo, F., Gilbin, R., Bourrachot, M.F.S., Morello, J.G.-L.M., 2006. Effects of chronic internal alpha irradiation on physiology, growth and reproductive success of *Daphnia magna*. Aquat. Toxicol. 80, 228–236.

Austad, S.N., 2009. Is there a role for new invertebrate models for aging research? J. Gerontol. A 64, 192–194.

Backlund, M.G., Trasti, S.L., Backlund, D.C., Cressman, V.L., Godfrey, V., Koller, B.H., 2001. Impact of ionizing radiation and genetic background on mammary tumorigenesis in p53-deficient mice. Cancer Res. 61, 6577–6582.

Barzilai, A., Yamamoto, K., 2004. DNA damage responses to oxidative stress. DNA Repair 3, 1109–1115.

Boëchat, I.G., Adrian, R., 2006. Evidence for biochemical limitation of population growth and reproduction of the rotifer *Keratella quadrata* fed with freshwater protists. J. Plankton Res. 28, 1027–1038.

Bozovic, V., Enesco, H.E., 1986. Effect of antioxidants on rotifer lifespan and activity. Age 9, 41–45.

Bradford, M.M.A., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 7, 248–254.

Cassidy, C.L., Lemon, J.A., Boreham, D.R., 2007. Impacts of low-dose gamma-radiation on genotoxic risk in aquatic ecosystems. Dose–Response 5, 323–332.

Dahms, H.-U., Hagiwara, A., Lee, J.-S., 2011. Ecotoxicology, ecophysiology, and mechanistic studies with rotifers. Aquat. Toxicol. 101, 1–12.

Driver, C.J., 1994. Ecotoxicity Literature Review of Selected Hanford Site Contaminants. PNL-9394. Pacific Northwest Lab, Richland, WA, USA.

Drotschmann, K., Hall, M.C., Shcherbakova, P.V., Wang, H., Erie, D.A., Brownewel, F.R., Kool, E.T., Kunkel, T.A., 2005. DNA binding properties of the yeast Msh2-Msh6 and Mlh1-Pms1 heterodimers. Biol. Chem. 383, 969–975.

Du, X.-I., Jiang, T., Wen, Z.-Q., Gao, R., Cui, M., Wang, F., 2009. Silencing of heat shock protein 70 expression enhances radiotherapy efficacy and inhibits cell invasion in endometrial cancer cell line. Croat. Med. J. 50, 143–150.

Dubrova, Y.E., Nesterov, V.N., Krouchinsky, N.G., Ostapenko, V.A., Neumann, R., Neil, D.L., Jeffreys, A.J., 1996. Human minisatellite mutation rate after the Chernobyl accident. Nature 380, 683–686.

Essers, J., Hendriks, R.W., Swagemakers, S.M.A., Troelstra, C., deWit, J., Bootsma, D., Hoeijimakers, J.H.J., Kanaar, R., 1997. Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. Cell 89, 195–204.

Fischbein, A., Zabludovsky, N., Eltes, F., Grischenko, V., Bartoov, B., 1997. Ultramorphological sperm characteristics in the risk assessment of health effects after radiation exposure among salvage workers in Chernobyl. Environ. Health Perspect. 105, 1445–1449.

Fisher, N.S., Beaugelin-Seiller, K., Hinton, T.G., Baumann, Z., Madigan, D., Garnier-Laplace, J., 2013. Evaluation of radiation doses and associated risk from the Fukushima nuclear accident to marine biota and human consumers of seafood. Proc. Natl. Acad. Sci. U. S. A. 110, 10670–10675.

Fuma, S., Ishii, N., Takeda, H., Doi, K., Kawaguchi, I., Shikano, S., Tanaka, N., Inamori, Y., 2010. Effects of acute γ-irradiation on community structure of the aquatic microbial microcosm. J. Environ. Radioactiv. 101, 915–922.

Garnier-Laplace, J., Beaugelin-Seiller, K., Hinton, T.G., 2011. Fukushima wildlife dose reconstruction signals ecological consequences. Environ. Sci. Technol. 45, 5077–5078.

Greenwood, L.N., Knowles, J.F., 1995. Effect of chronic irradiation on the humoral immune response of a marine fish, the eelpout (*Zoarces viviparus* L.). Int. J. Radiat. Biol. 67, 71–77.

Groth, P., Orta, M.L., Elvers, I., Majumder, M.M., Lagerqvist, A., Helleday, T., 2012. Homologous recombination repairs secondary replication induced DNA double-strand breaks after ionizing radiation. Nucleic Acids Res. 40, 6585–6594.

Guo, S., Zhang, Y., Yuan, F., Gao, Y., Gu, L., Wong, I., Li, G.-M., 2006. Regulation of replication protein A functions in DNA mismatch repair by phosphorylation. J. Biol. Chem. 281, 21607–21616.

Han, J., Won, E.-J., Hwang, D.-S., Rhee, J.-S., Kim, I.-C., Lee, J.-S., 2013. Effect of copper exposure on GST activity and on the expression of four GSTs under oxidative stress condition in the monogonont rotifer, *Brachionus koreanus*. Comp. Biochem. Physiol. C 158, 91–100.

Han, J., Won, E.-J., Kim, I.-C., Yim, J.H., Leung, K.M.Y., Lee, Y.S., Lee, J.-S., 2014. Gamma ray induces DNA damage and oxidative stress with impaired growth and reproduction in the copepod *Tigriopus japonicus*. Aquat. Toxicol. 152, 264–272.

Haney, J.D., Jackson, G.A., 1996. Modeling phytoplankton growth rates. J. Plankton Res. 18, 63–85.

Hartlerode, A., Odate, S., Shim, I., Brown, J., Scully, R., 2011. Cell cycle-dependent induction of homologous recombination by a tightly regulated I-SceI fusion protein. PLoS ONE 6, e16501.

Hwang, D.-S., Suga, K., Sakakura, Y., Park, H.G., Hagiwara, A., Rhee, J.-S., Lee, J.-S., 2013. Complete mitochondrial genome of the monogonont rotifer, *Brachionus koreanus* (Rotifera, Brachionidae). Mitochondr. DNA 25, 29–30.

Imai, J., Yashiroda, H., Maruya, M., Yahara, I., Tanaka, K., 2003. Proteasomes and molecular chaperones. Cell Cycle 2, 585–589.

Jennings, J.S., Lynch, R.S., 1928. Age, mortality, fertility and individual diversities in the rotifer *Proalessordida* Gosse. II. Life history in relation to mortality and fecundity. J. Exp. Zool. 51, 339–381.

Johnson, T.E., Hartman, P.S., 1988. Radiation effects on life span in *Caenorhabditis elegans*. J. Gerontol. 43, 137–141.

Jung, M.-Y., Lee, Y.-M., 2012. Expression profiles of heat shock protein gene families in the monogonont rotifer *Brachionus koreanus*-exposed to copper and cadmium. Toxicol. Environ. Health Sci. 4, 235–242.

Kameník, J., Dulaiova, H., Buesseler, K.O., Pike, S.M., Šťastná, K., 2013. Cesium-134 and137 activities in the ventral North Pacific Ocean after the Fukushima Dai-ichi nuclearpower plant accident. Biogeosciences 10, 5223–5244.

Kang, M.A., So, E.-Y., Simons, A.L., Spitz, D.R., Ouchi, T., 2012. DNA damage induces reactive oxygen species generation through the H2AX-Nox1/Rac1 pathway. Cell Death Dis. 3, e249.

Keister, J.E., Bonnet, D., 2012. Zooplankton population connections, community dynamics, and climate variability. ICES J. Mar. Sci. 69, 347–350.

Kemp, C.J., Wheldon, T., Balmain, A., 1994. p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. Nat. Genet. 8, 66–69.

Kim, R.-O., Rhee, J.-S., Won, E.-J., Lee, K.-W., Kang, C.-M., Lee, Y.-M., Lee, J.-S., 2011. Ultraviolet B retards growth, induces oxidative stress, and modulates DNA repair-related gene and heat shock protein gene expression in the monogonont rotifer, *Brachionus* sp. Aquat. Toxicol. 101, 529–539.

Kitamura, M., Kumamoto, Y., Kawakami, H., Cruz, E.C., Fujikura, K.B.G.D., 2013. Horizontal distribution of Fukushima-derived radiocesium in zooplankton in the northwestern Pacific Ocean. Biogeosciences 10, 5729–5738.

Knowles, J.F., 1992. The effect of chronic radiation on the humoral immune response of rainbow trout (*Onchorhynchus mykiss* Walbaum). Int. J. Radiat. Biol. 62, 239–248.

Knowles, J.F., 1999. Long-term irradiation of a marine fish, the plaice *Pleuronectes platessa*: an assessment of the effects on size and composition of the testes and of possible genotoxic changes in peripheral erythrocytes. Int. J. Radiat. Biol. 75, 773–782.

Kobayashi, T., Takeuchi, S., Saijo, M., Nakatsu, Y., Morioka, H., Otsuka, E., Wakasugi, M., Nikaido, O., Tanaka, K., 1998. Mutational analysis of a function of *Xeroderma pigmentosum* group A (XPA) protein in strand-specific DNA repair. Nucleic Acids Res. 26, 4662–4668.

Kovalchuk, O., Kovalchuk, I., Titov, V., Arkhipov, A., Hohn, B., 1999. Radiation hazard caused by the Chernobyl accident in inhabited areas of Ukraine can be monitored by transgenic plants. Mutat. Res. 446, 49–55.

Krisko, A., Leroy, M., Radman, M., Meselson, M., 2012. Extreme anti-oxidant protection against ionizing radiation in bdelloid rotifers. Proc. Natl. Acad. Sci. U. S. A. 109, 2354–2357.

Krishnaswami, S., Baskaran, M., Fowler, S.W., Heyraud, M., 1985. Comparative role of salps and other zooplankton in the cycling and transport of selected elements and natural radionuclides in Mediterranean waters. Biogeochemistry 1, 353–360.

Krivolutzkii, D.A., Pokarzhevskii, A.D., 1992. Effects of radioactive fallout on soil animal populations in the 30 km zone of the Chernobyl atomic power station. Sci. Total Environ. 112, 69–77.

Kumamoto, T., Murata, A., Kawano, T., Aoyama, M., 2013. Fukushima-derived radiocesium in the northwestern Pacific Ocean in February 2012. Appl. Radiat. Isotope 81, 335–339.

Lee, S.-J., Choi, S.-A., Lee, K.-H., Chung, H.-Y., Kim, T.-H., Cho, C.-K., Lee, Y.-S., 2001. Role of inducible heat shock protein 70 in radiation-induced cell death. Cell Stress Chaperones 6, 273–281.

Lee, J.-S., Kim, R.-O., Rhee, J.-S., Han, J., Hwang, D.-S., Choi, B.-S., Lee, C.J., Yoon, Y.-D., Lim, J.-S., Lee, Y.-M., Park, G.S., Hagiwara, A., Choi, I.-Y., 2011. Sequence analysis of genomic DNA (680 Mb) by GS-FLX-Titanium sequencer in the monogonont rotifer, *Brachionus ibericus*. Hydrobiologia 662, 65–75.

Liu, V., Weaver, D.T., 1993. The ionizing radiation-induced replication protein a phosphorylation response differs between ataxia telangiectasia and normal human cells. Mol. Cell. Biol. 13, 7222–7231.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta C}$t method. Methods 25, 402–408.

Marques, M., Sousa, A.P., Paiva, A., Almeida-Santos, T., Ramalho-Santos, J., 2014. Low amounts of mitochondrial reactive oxygen species define human sperm quality. Reproduction 147, 817–824.

Mazin, A.V., Alexeev, A.A., Kowalczykowski, S.C., 2003. A novel function of Rad 54 protein. Stabilization of the Rad 51 nucleoprotein filament. J. Biol. Chem. 18, 14029–14036.

Miccoli, L., Burr, K.L.A., Hickenbotham, P., Friedberg, E.C., Angulo, J.F., Dubrova, Y.E., 2007. The combined effects of *Xetoderma pigmentosum* C deficiency and mutagens on mutation rates in the mouse germ line. Cancer Res. 67, 4695–4699.

Mitani, H., Egami, N., 1982. Rejoinig of DNA strand breaks after gamma-irradiation in cultured fish cells, CAF-MM1. Int. J. Radiat. Biol. 41, 85–90.

Moreno-Garrido, I., Lubián, L.M., Soares, A.M.W.M., 1999. In vitro populations of rotifer *Brachionus plicatilis* Müller demonstrate inhibition when fed with copper-preaccumulating microalgae. Ecotoxicol. Environ. Saf. 44, 220–225.

Myers, D.K., 1989. The General Principles and Consequences of Environmental Radiation Exposure in Relation to Canada's Nuclear Fuel Waste Management Concept. AECL 9917. Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Chalk River, Ontario, Canada.

Nollen, E.A., Morimoto, R.I., 2002. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. J. Cell Sci. 115, 2809–2816.

Park, S.-H., Lee, S.-J., Chung, H.-Y., Kim, T.-H., Cho, C.-K., Yoo, S.-Y., Lee, Y.-S., 2000. Inducible heat shock protein 70 involved in the radioadaptive response. Radiat. Res. 153, 318–326.

Parsells, D.A., Lindquist, S., 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet. 27, 437–496.

Petes, L.E., Menge, B.A., Murphy, G.D., 2007. Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. J. Exp. Mar. Biol. Ecol. 351, 83–91.

Rhee, J.-S., Kim, B.-M., Kang, C.-M., Lee, Y.-M., Lee, J.-S., 2012. Gamma irradiation-induced oxidative stress and developmental impairment in the hermaphroditic fish, *Kryptolebias marmoratus* embryo. Environ. Toxicol. Chem. 31, 1745–1753.

Rhee, J.-S., Kim, B.-M., Kim, R.-O., Seo, J.S., Kim, I.-C., Lee, Y.-M., Lee, J.-S., 2013. Co-expression of antioxidant enzymes with expression of *p53*, DNA repair, and heat shock protein genes in the gamma ray-irradiated hermaphroditic fish *Kryptolebias marmoratus* larvae. Aquat. Toxicol. 140/141, 58–67.

Riley, P.A., 1994. Free radicals in biology: oxidative stress and the effects of ionizing radiation. Int. J. Radiat. Biol. 65, 27–33.

Shahi, A., Lee, J.H., Kang, Y., Lee, S.H., Hyun, J.W., Chang, I.Y., Jun, J.Y., You, H.J., 2011. Mismatch-repair protein MSH6 is associated with Ku70 and regulates DNA double-strand break repair. Nucleic Acids Res. 39, 2130–2143.

Snell, T.W., King, C.E., 1977. Lifespan and fecundity patterns in rotifers: the cost of reproduction. Evolution 31, 882–890.

Spagnolo, L., Rivera-Calzada, A., Pearl, L.H., Llorca, O., 2006. Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. Mol. Cell 22, 511–519.

Stewart, G.M., Fowler, S.W., Teyssie, J.L., Cotret, O., Cochran, J.K., Fisher, N.S., 2005. Contrasting the transfer of polonium-210 and lead-210 across three trophic levels in the marine plankton. Mar. Ecol. Prog. Ser. 290, 27–33.

Vispé, S., Cazaux, C., Lesca, C., Defais, M., 1998. Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. Nucleic Acids Res. 26, 2859–2864.

Ward, J.F., Kuo, I., 1976. Strand breaks, base release, and post irradiation changes in DNA γ-irradiated in dilute O_2^- saturated aqueous solution. Radiat. Res. 66, 485–498.

Westmoreland, T.J., Marks, J.R., Olson Jr., J.A., Thompson, E.M., Resnik, M.A., Bennett, C.B., 2004. Cell cycle progression in G1 and S phases is CCR4 dependent following ionizing radiation or replication stress in *Saccharomyces cerevisiae*. Eukaryot. Cell 3, 430–446.

Won, E.-J., Lee, J.-S., 2014. Gamma radiation induces growth retardation, impaired egg production, and oxidative stress in the marine copepod *Paracyclopina nana*. Aquat. Toxicol. 150, 17–26.

Woodhead, D.S., 1977. The effects of chronic irradiation on the breeding performance of the guppy *Poecilia reticulata* (Osteichthyes: Teleostei). Int. J. Radiat. Biol. 32, 1–22.

Yoshinaga, T., Hagiwara, A., Tsukamoto, K., 2001. Why do rotifer populations present a typical sigmoidal growth curve? Hydrobiologia 446/447, 99–105.

Yúfera, M., Navarro, N., 1995. Population growth dynamics of the rotifer *Brachionus plicatilis* cultured in non-limiting food condition. Hydrobiologia 313/314, 399–405.

Zaka, R., Chenal, C., Misset, M.T., 2004. Effects of low doses of short-term gamma irradiation on growth and development through two generations of *Pisum sativum*. Sci. Total Environ. 320, 121–129.