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Physiological Responses of Intrinsic Small Abalone *Haliotis diversicolor aquatilis* under High Temperature Stress by Low Level ^{60}CO Gamma Irradiation-Mediated Hormetic Effect

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Abstract: *Haliotis diversicolor aquatilis (H. diversicolor aquatilis)* is one of the aquaculture abalone species in Jeju Island, South Korea. The high water temperature in the middle of the summer season frequently limits the aquaculture productivity of abalone. To cope with the problem, this study aimed to investigate viability, attachment rate, changes of immune response, and physiological properties of juvenile small abalone *Haliotis diversicolor aquatilis* against high water temperature stress under ^{60}CO-gamma irradiation-mediated hormetic effects. To examine physiological changes of abalone, the gamma-ray irradiated and non-irradiated groups were distinguished by grouping a total of 70 abalones in a treated group and a control group (each group included twenty-five female and ten male abalones). The treated group was exposed to ^{60}CO-gamma irradiation using a designed experimental apparatus, the control group was not. Our results revealed that the low level of gamma ray (20 Gy)-irradiated *Haliotis diversicolor aquatilis* showed a 100% survival rate during the experiment. After gamma ray exposure, all the abalones were cultured without feeding and bioactivities were measured to examine gamma ray-induced physiological responses. The results suggested the potential for selective breeding using gamma ray irradiation hormesis to manipulate the number of eggs, fertilization rate, hatching rate, and attachment rate. The shell length of juvenile abalone was significantly enhanced by a 20 Gy radiation dose. We could presume that the effect of hormesis in the gamma-ray irradiation parent shellfish also had a genetic effect on the offspring. In order to verify changes in immune response and stress tolerance of abalone under high temperature stress, lysozyme activity and survival rates were compared at a water temperature of 30 °C. Interestingly, the ^{60}Co gamma ray-irradiated abalones exhibited almost a 1.65-fold enhanced survival rate along with reduced lysozyme activity after 12 h of high temperature stress. Our results speculate that low levels of ^{60}Co gamma ray-mediated hormetic effects can be an effective strategy for shell length growth and high temperature stress tolerance.

Keywords: ^{60}CO-gamma irradiation; *Haliotis diversicolor aquatilis*; abalone hormesis; mutation induction; Jeju Island

1. Introduction

Hormesis can be explained as an adaptation to harsh changing environments. The bioactive mechanism of hormesis has yet to be established. Several hypotheses have been suggested based on studies of land animals and plants. The low dose radiation produces proteins related to the DNA recovery process while the DNA synthesis process is temporarily inhibited, providing sufficient time for the treated cells to recover. At the same time, resistance is increased when free radicals spread [1].

Low-dose radiation treatment technology such as gamma radiation may be used to induce hormesis in plant species including cabbage, pepper, and gourd [2,3] to stimulate growth, increase viability and productivity, stabilize food supply, and increase plant resistance to environmental stressors [4]. Hormesis and increased growth has also been reported for the marine abalone species, *Haliotis discus hannai* and *Haliotis discus* at gamma-ray doses of 30 Gy and 20 Gy respectively [5,6], however, no studies have been undertaken for *Haliotis diversicolor aquatilis*.

The bioactive mechanism of hormesis has yet to be established, but several possibilities are being presented. The low dose radiation produces proteins related to the DNA recovery process while the DNA synthesis process is temporarily inhibited, providing sufficient time for the targeted cells to recover. At the same time, resistance is increased when free radicals spread [1]. These theories were established based on the experimental results of land animals and plants, but in the case of *Haliotis diversicolor aquatilis*, which is marine life, there are few studies.

Haliotis diversicolor aquatilis is a species that only inhabits East Asia such as China and Jeju Island, Korea. It has quite a high industrial value. Climate change due to global warming has recently caused physiological changes such as growth, breeding, metabolic, and osmotic pressure, which have caused problems such as disease and death in abalone aquaculture. In particular, in the summer of 2006, low salinity seawater from China flowed into the northwestern coast of Jeju Island, causing the salinity to drop below 20 psu (practical salinity unit: ‰). With this phenomenon, fishermen encountered the massive death of abalone, resulting in economic losses of up to $5 million (six billion Korean won). In general, most abalones of Korea have a long growth period and have diverse growth variables due to environmental changes during this time. Stable abalone production requires an understanding of the factors that affect growth and survival [7,8]. Up to this day, aquaculture scientists have researched the correlation between *Haliotis diversicolor aquatilis* physiology and environmental changes [9]. Some researchers have shown that *Haliotis diversicolor aquatilis*, *Haliotis discus*, and *Haliotis sieboldi* in Jeju Island are subject to physiological changes due to stress induced by seawater temperature [9,10].

Studies regarding breeding mostly address topics of growth traits and genetic parameter estimation [11], but there is almost no research on active breed improvement. Methods used for plant breeding are widely used such as selective breeding, hybrid breeding, and mutation breeding by irradiation. In particular, there have been many studies on hormesis using radiation, but few studies using radiation for breed improvement. In this study, gamma-ray hormesis was induced to *Haliotis diversicolor aquatilis*, a flagship aquaculture species of Jeju Island. After gamma irradiation, basic physiological changes in juvenile small abalone such as attachment rate, growth rate, and immunoactivity change according to water temperature stress were recorded and analyzed to determine if 20 Gy-irradiated *Haliotis diversicolor aquatilis* exhibited increased growth rates.

2. Materials and Methods

2.1. Animals

For the present study, we purchased *Haliotis diversicolor aquatilis* from Jeju City fishing village societies on Jeju Island, Korea. Samples were acclimated to the experimental setup one week in a tank at 23 ± 0.3 °C and 32 ± 0.2 psu (practical salinity unit: ‰) of salinity without feeding. In all, 100 female and 20 male *Haliotis diversicolor aquatilis* were used for the experiment. The average shell lengths (mean ± standard deviation) of *Haliotis diversicolor aquatilis* were 6.25 ± 0.2 (range 6.05–6.45), and the average total wet weights were 32.5 ± 1.5 g (range, 31–34 g). The twenty-five (25) females and ten (10) males were exposed to each gamma radiation dose (0 and 20 Gy).

2.2. Fabrication of the Case for Gamma-Ray Irradiation Use

Haliotis diversicolor aquatilis is a marine invertebrate that dies if exposed to air for a long time. It shows characteristics of being mobile and lives by attaching to something else. To induce mutations by gamma irradiation, a case was made to minimize the mobility limitations of *Haliotis diversicolor aquatilis* and the stress during the attachment and desorption of *Haliotis diversicolor aquatilis*. The case was a hollow acrylic box with a width of 10 cm, a length of 10 cm, and a height of 5 cm. The front surface was made detachable to facilitate the attachment and desorption of the *Haliotis diversicolor aquatilis*. For this, grooves were formed on both sides of the acrylic box to facilitate the separation of the front plate (Figure 1).

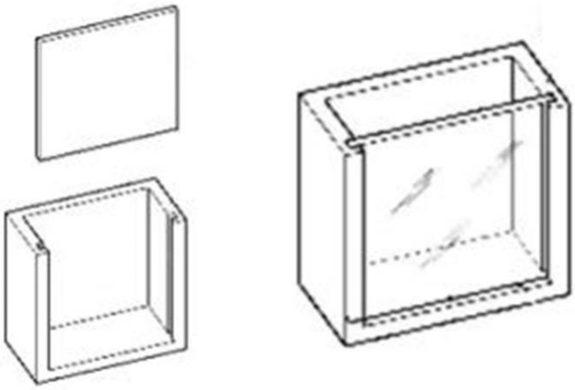


Figure 1. Case for irradiation with gamma-rays [2].

2.3. ^{60}CO Gamma Irradiation

As abalone species have adhesive and migratory properties and perish after long-term exposure to air, we designed small acrylic cases for the experiment. The cases were 10 cm wide, 10 cm long, and 5 cm high. To facilitate the disadhesion of abalone species, we made the cases with a detachable acrylic panel (Figure 1). We conducted gamma radiation (GR) exposure experiments at the Institute for Nuclear Science and Technology at Jeju National University, Korea with ^{60}CO gamma irradiation (10,000 ci), as shown in Figure 2. GR was set at 0 and 20 Gy by regulating the irradiation time to 0 and 36 min, respectively. The radiation level was measured using a DE/FH40G-L survey meter (Eberline, TN, USA). *Haliotis diversicolor aquatilis* was exposed to all of the radiation levels stated above and GR-exposed abalone groups were incubated under optimal conditions (23 ± 0.3 °C and 32 ± 0.2 psu of salinity) without feeding for six weeks.

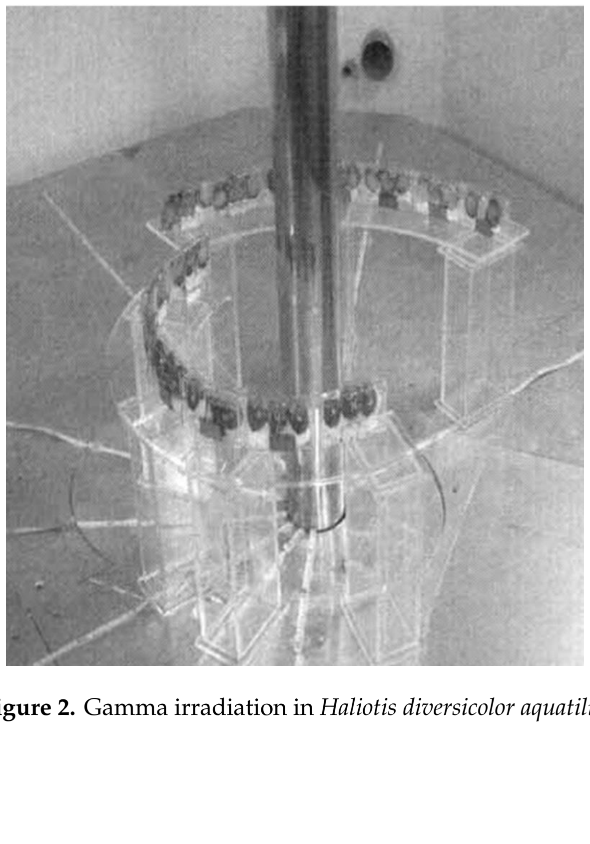


Figure 2. Gamma irradiation in *Haliotis diversicolor aquatilis*.

2.4. Seed Production of Haliotis Diversicolor Aquatilis after Gamma-Ray Irradiation

Both *Haliotis diversicolor aquatilis* parents of the gamma irradiated and unirradiated samples were acclimatized at optimal water temperature (23 ± 0.3 °C) for a week, before conducting artificial seed production. After 1 h of excitation stimulation at 30 °C, UV seawater stimulation was performed to induce artificial spawning of samples. In gamma-ray-irradiated samples and unirradiated experimental control group samples, the mixing of the eggs with the sperm were separately performed to prevent mixing fertilized eggs from each group. The fertilized eggs were collected using 100 μm muller gauze, then the fertilized embryo population was counted.

2.5. Fertilization Rate and Hatching Rate Count

The fertilization rate was counted and calculated with about 200,000 samples of fertilized eggs of the gamma irradiated group and an unirradiated experimental control group by separately transferring the fertilized eggs from a 1 mL glass beaker to a 50 mL glass beaker in triplicate. Then, after putting the fertilized eggs in a 20 L circular plastic container, the hatched larvae and the unhatched larvae were collected and moved to the 1 L glass beaker and the overall hatching rate was measured by pipetting 1 mL of the fertilized eggs in triplicate.

2.6. Larvae Management

The fertilized eggs of *Haliotis diversicolor aquatilis* were accommodated in two 4-ton tanks of fiber reinforced plastic (1 m width × 6 m length × 70 cm height) with one water tank for each experimental group at 24 ± 0.5 °C. After the veliger larvae were fully formed, the larvae were raised to the surface using the air supply and water flow systems. Seeds were collected by gathering the larvae on the shelter plate in which diatoms were cultured in advance.

2.7. Changes in Attachment Rate and Growth Rate

The attachment rate was measured by counting *Haliotis diversicolor aquatilis* attached to the shelter plate by randomly selecting three shelter plates from the shelter plate set (30 cm width × 30 cm length), which were separated by seed collection dosage. The growth rate was measured by the size of three randomly selected *Haliotis diversicolor aquatilis* attached to the water tank shelter plate of each experimental group using Vernier calipers 90 days after hatching when the abalones could clearly be identified by the naked eye.

2.8. Immune-Activity Changes of Small Abalones Due to Water Temperature Stress

For the immuno-activity changes, small abalones that were produced from the control and 20 Gy were bred for 48 weeks, and subsequently, 30 similar small abalones were selected from each group. Small abalones were used with an average shell length of 2.48 ± 0.05 cm, shell width of 1.21 ± 0.05 cm, and a total weight of 2.1 ± 0.3 g. To determine whether the immune-activity increased, five abalone were collected when the sudden change of water temperature was at 0 h and 12 h, respectively, using the pre-studied temperature of 30 °C (100% of the dead-water temperature and lysozyme activity change was evaluated). The blood was collected from the blood vessels next to the heart of *Haliotis diversicolor aquatilis* using a 1 mL syringe with a needle standard of 26 GX 1/2 and diluted tenfold with a 0.9% saline solution. The lysozyme activity inside the serum was analyzed by a turbidimetric assay according to the following methods: *Micrococcus lysodeikticus* (0.1 mg/mL PBS, pH 6.8) suspension 90 μL, and serum 100 μL were mixed in a 96-well plate and reacted for 5 min in a 25 °C culture medium, then the absorbance was measured at 530 nm. Lysozyme activity was marked as a 0.001/mL decrease in absorbance values per 1 unit.

2.9. Statistical Tool

Statistical analysis was processed using a one-way analysis of variances (ANOVA) followed by post-hoc t-tests by using an IBM SPSS 22 program. The values of ($p < 0.05$) were considered significant, and we experiments were conducted in triplicate.

3. Results and Discussion

3.1. Survival Rate of Parent Shellfish and Fertilization Rate

The dam (mother) samples of irradiated 20 Gy gamma-ray showed dull activity until the time of domestication and egg collection, but indicated a survival rate of 100%. The control group and the dams that were exposed to irradiated 20 Gy of gamma-ray were used to create seed production. Each of the spawned eggs were separated and fertilized.

The number of eggs spawned in each experimental group was measured by an optical microscope (Nikon PROFILE PROJECTOR V-12B) using a 1 mL pipette, and the results are shown in Figure 3. The fertilized eggs of 2,000,000 ± 130 were found in the control group, and 1,000,000 ± 110 fertilized eggs were found in the 20 Gy experimental group.

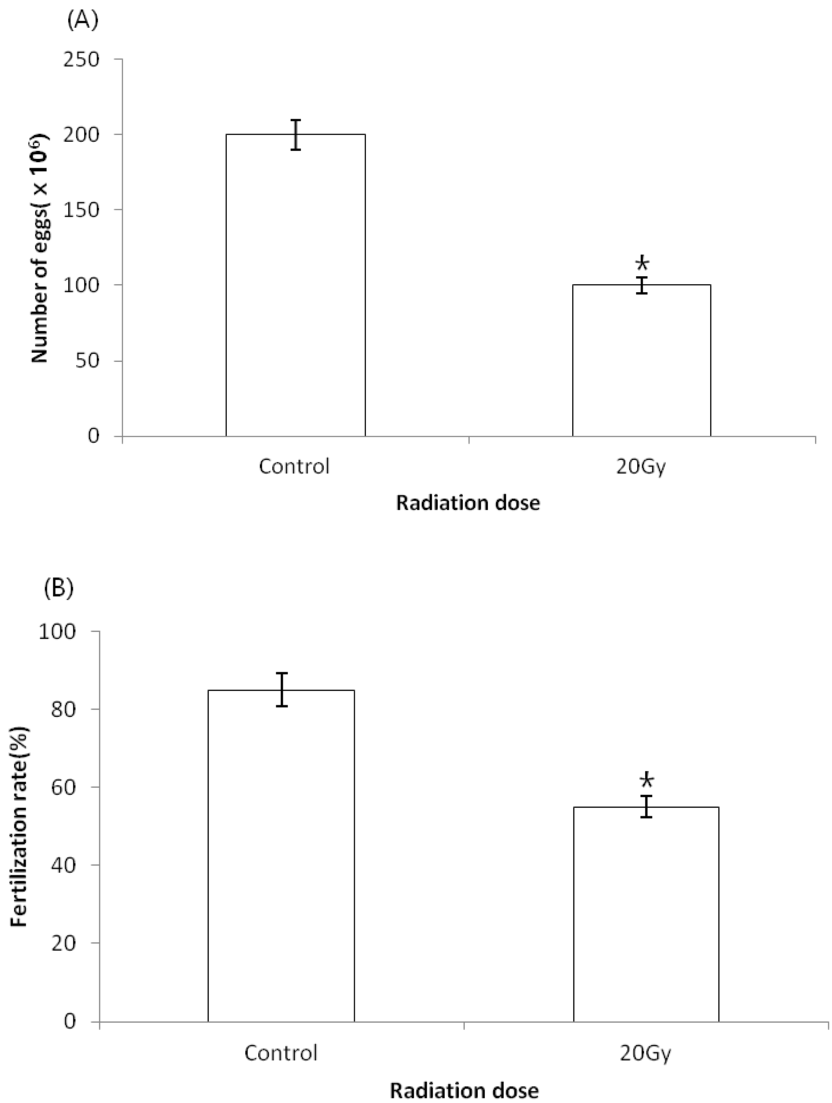


Figure 3. Number of eggs (**A**) and fertility (**B**) for each gamma-ray dose. * $p < 0.05$ for control.

In order to obtain accurate results, about 200,000 fertilized eggs were collected. The fertilized and non-fertilized eggs in each experimental group were counted, and as a result of examining the fertilized eggs and fertilization rates according to gamma irradiation, 185,000 ± 170 samples of fertilized eggs were found in the experimental control group, and 110,000 ± 150 samples of fertilized eggs were found in the 20 Gy experimental group. The fertilization rate was confirmed as 85% in the experimental control group and 55% in the 20 Gy experimental group. In the mean water temperature of 24 °C, it took 8 h from fertilization to hatching, 16 h until formation of the young shell, and 56 h until the seedling collection stage. There was no significant difference between the experimental groups ($p < 0.05$).

3.2. Hatching Rate and Attachment Rate Measurement

In order to measure the hatching and attachment rate, fertilized eggs were reared in the sea water tank for each experimental group. The results are shown in Figure 4. The hatching rate was indicated as 70 ± 5% in the control and 30 ± 5% in the 20 Gy exposed group. The attachment rate was revealed as 80 ± 5% in the control and 65 ± 5% in the 20 Gy exposed group. The number of fertilized eggs, fertilization rate, hatching rate, and attachment rate was lower in the 20 Gy experimental group than in the control group (where the number of small abalone was low ($p < 0.05$)).

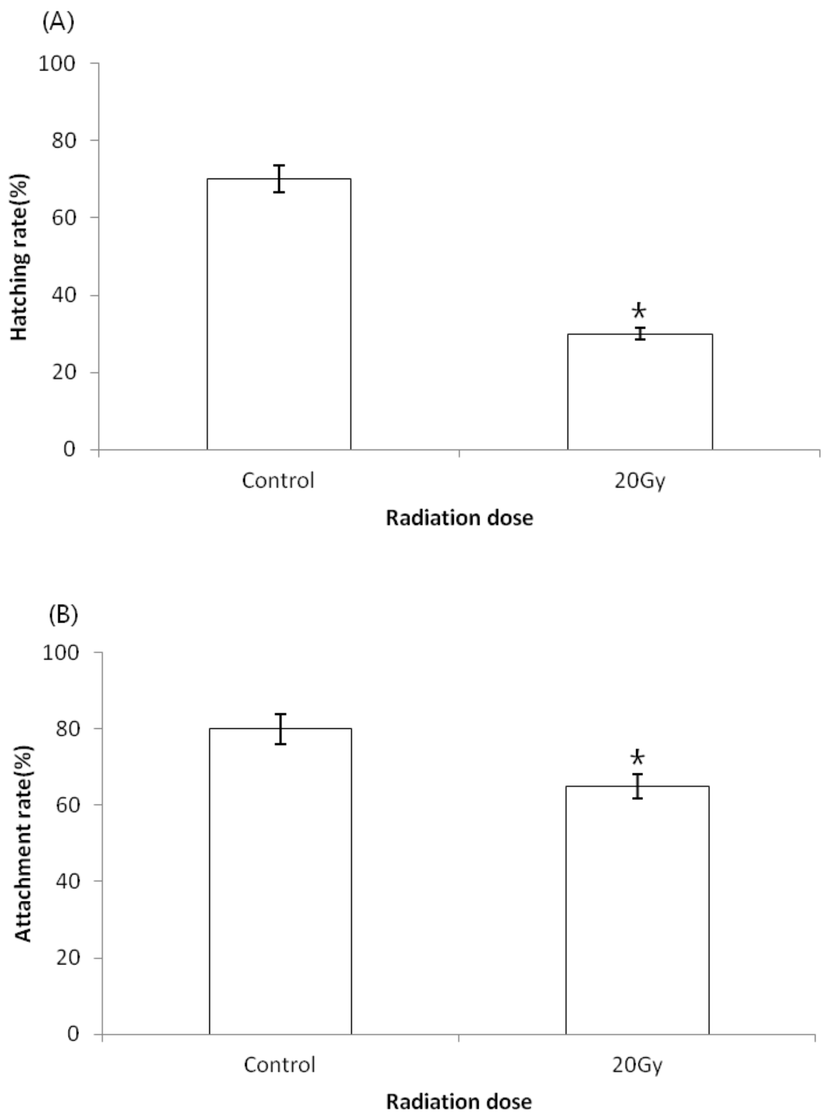


Figure 4. Hatching rate (**A**) and attachment rate (**B**) for each gamma-ray dose. * $p < 0.05$ for control.

3.3. Growth Measurement of Small Abalones

Thirty randomly selected *Haliotis diversicolor aquatilis* attached to each experimental group's shelter plate in the water tank was measured after 90 days, as demonstrated in Figure 5. A total of 65 shelter plates and 10 abalones per shelter plate were attached. An average of 650 ± 70 small abalones were attached. The average temperature of the aquaculture farm was 25.6 °C and the average salinity was 32.6 psu. The general natural condition of the photoperiod was 10 L:14 D. The control group grew by 1.1 ± 0.5 mm and the 20 Gy group grew by 1.6 ± 0.5 mm ($p < 0.05$).

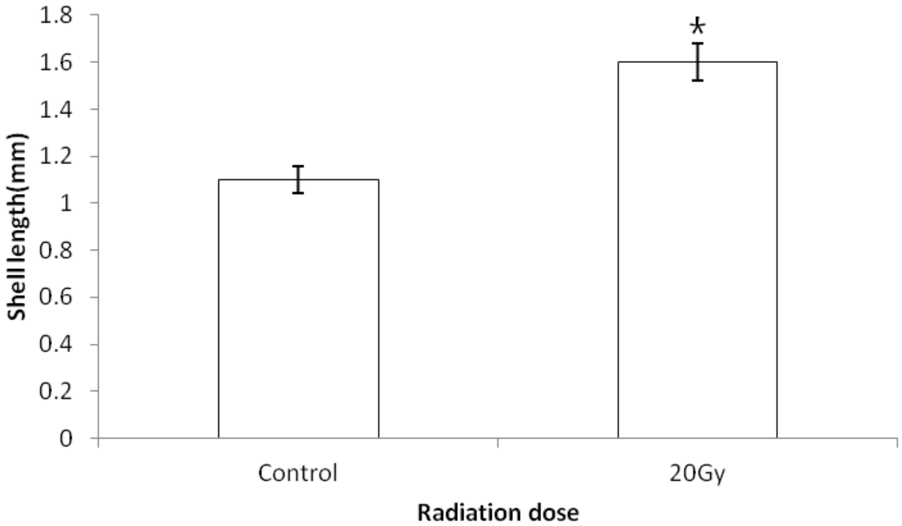


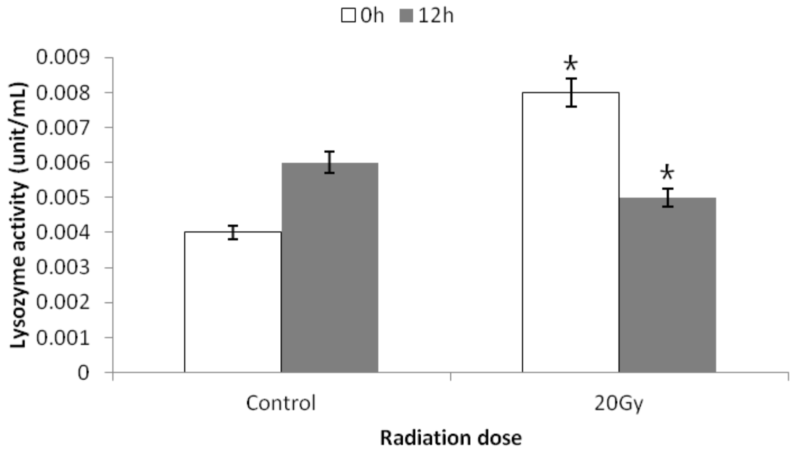
Figure 5. Changes in growth in juvenile *Haliotis diversicolor aquatilis* for each gamma-ray dose.

3.4. Changes of Immune-Activity by Water Temperature Stress

To investigate the cause of the high growth rate after gamma irradiation, *Haliotis diversicolor aquatilis* stress was investigated under a high water temperature of 30 °C. The lysozyme activity change is an environmental stress factor that is used as an indicator of immune function, as shown in Figure 6. The lysozyme activity change in the treated group of 20 Gy was shown to be significantly higher at 0 h than at 12 h under the 30 °C temperature water ($p < 0.05$). Additionally, the survival rate resulted in terms of immune-activity after 12 h are described in Figure 7. This indicates the survival rate of 34% in the control group and 56% in the 20 Gy group ($p < 0.05$).

Many studies of breeding through mutation induction have been conducted focusing on agriculture and its effect and economic value [12,13]. In this study, low-dose gamma-rays were irradiated to parent shellfish of *Haliotis diversicolor aquatilis*. Their growth was observed through physiological changes and breeding. The next generation of *Haliotis diversicolor aquatilis* produced after gamma irradiation was also observed. Generally, it is reported that gamma ray irradiation causes oxidative stress in cells caused by the water molecule's overproduction of hydroxyl [14]. The results of this study illustrated that there were no deaths of parent shellfish in the high concentration gamma-ray irradiation section of 20 Gy, but a phenomenon of decreased activity emerged. Therefore, the dose of gamma-ray set in this study may cause some stress on parent shellfish, but does not affect breeding, so was considered suitable for mutation induction.

It was pointed out that the hatching rate of fertilized eggs produced in the control group was 70 ± 5% and the attachment rate was 80 ± 5%, the hatching rate of fertilized eggs produced in the 20 Gy exposed group was 30 ± 5%, and the attachment rate was 65 ± 5%. The experimental group of 20 Gy showed a low number of fertilized eggs, fertilization rate, hatching rate, and attachment rate, so it resultingly indicated a lower number of small abalones than the control group. According to Kim et al. [15], it is reported that gamma-ray irradiation in younger abalone caused stronger DNA and RNA damage and caused physiological disorders. The reduction of the hatching and attachment rate at high doses examined in this study was also presumed to be a result of concentration, presumably by causing gene damage to eggs existing in the body of the parent shellfish.



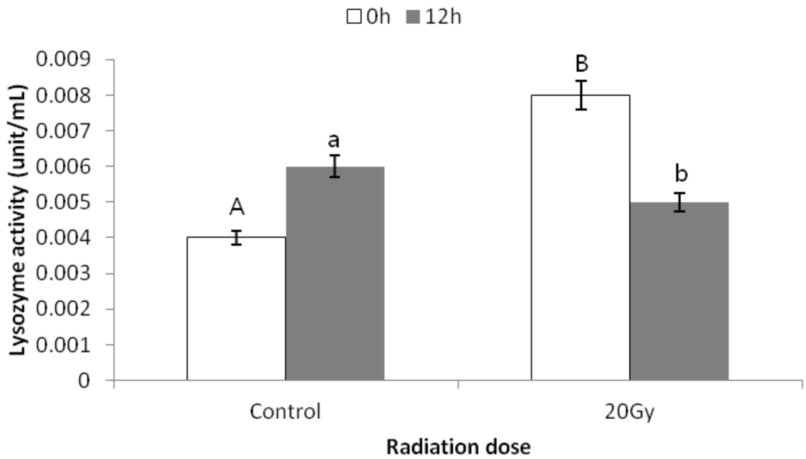


Figure 6. Effect of high water-temperature stress on abalone *Haliotis diversicolor aquatilis* lysozyme activity in the control or 20 Gy groups at a 0 h and 12 h incubation period.

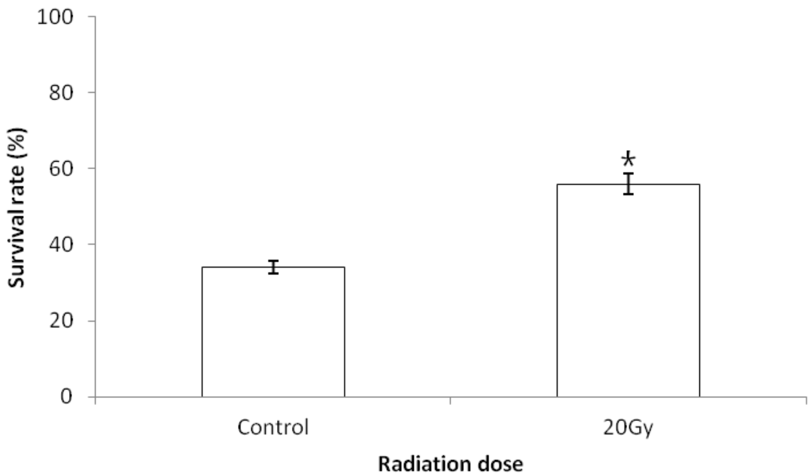


Figure 7. Survival of abalone *Haliotis diversicolor aquatilis* reared with different doses of radiation at 12 h after exposure to high-water temperature stress. * $p < 0.05$ for control.

Meanwhile, Lee et al. [16] observed increments of germination rates in a gamma-ray irradiated group and a control group. This observation focused on the resulting hormesis effect of irradiated low-dose gamma-rays on the seeds of chili crop according to each dose. The optimal doses affecting growth were reported between 1 Gy and 20 Gy. Kim et al. [17–19] also observed that the growth and quantity of corn, fruit, and cabbage from the irradiation of low dose gamma-rays to seeds increased. Koepp and Kramer [20] reported that increments of the germination rate and growth of low-dose gamma-ray irradiated corn affected the quantitative equilibrium of plant hormones, and that the low-dose gamma-ray, which penetrated the seed skin, eventually improved the seed skin's resistance to photosynthetic stress. Additionally, when the low dose gamma-ray irradiated *Haliotis diversicolor aquatilis* in this study, it showed a low hatching and attachment rate, but the growth was significantly high. This can be attributed to the gamma-ray irradiation affecting the growth and growth factors of the hormone system, which is similar to terrestrial plants. According to Kim et al. [21], water temperature and adaptability to low salinity environments were measured using F1 produced by hybridization of the *Pagrus major* (red seabream) and the *Acanthopagrus schlegelii* (black sea bream). It was reported that the fast-growing abalones are highly susceptible to variation in environmental changes. Invertebrate lysozymes are frequently used as an indicator of immune activity [22]. It has been known to play various roles in biodefense mechanisms by involving the opsonization and anti-parasitic action, antiviral action, and pollutant action, as it has cleansing and anti-inflammatory properties [23]. In this experiment, the lysozyme activity change from high water temperature stress was significantly greater in the gamma irradiation 20 Gy experimental group than in the control group at 0 h, and it was significantly lower than the control group at 12 h. Considering these results, the lysozyme activity was low in the control group without gamma-ray irradiation, while the lysozyme activity was high in the gamma-ray irradiation group. This suggests that the biodefense system against external stress was activated to remove harmful active oxygen, thereby showing a higher survival rate and rapid growth rate.

As a result of measuring the activity of lysozyme in *Haliotis diversicolor aquatilis* by the low salt stimulation, it was reported that the lysozyme activity was significantly increased and then decreased in other experimental groups compared to the control group immediately after administering stress (the same as in this study). These results suggest that the survival rate is improved by the increase in the initial immune-activity right after applying water temperature and salinity stress, and the abalones with strong resistance increase initial immune-activity, then stabilize rapidly.

4. Conclusions

The results of this study on the physiological effects of gamma irradiation using *Haliotis diversicolor aquatilis*, an invertebrate marine organism, revealed that there was a high survival rate, growth rate, and immune activity response in low dose gamma irradiation, as shown in the published results of existing studies of land plants. In other words, these results revealed the potential of selective breeding using gamma ray irradiation hormesis regarding the number of eggs, fertilization rate, hatching rate, and attachment rate. It is presumed that the effect of hormesis in the gamma-ray irradiation parent shellfish also has a genetic effect on the offspring. These results are expected to suggest the possibility of introducing new breeding techniques through the induction of gamma-ray mutation in marine organisms with a high mortality rate due to high water temperature such as scallops and sea squirts. However, physiological indicators are sensitive to various environmental changes, species, and individuals. It is deemed necessary to set up the evaluating indicators through a detailed experiment in the future.

Author Contributions: M.-s.J. and C.-Y.H. provided direction for the research work and participated in the research. C.-Y.H. did the literature review and collected relevant data and M.-s.J. wrote the manuscript. Additionally, M.-s.J. searched and collected data on irradiation experiment as well as searched and collected literature and evidence. C.-Y.H. revised the paper. M.-s.J. and C.-Y.H. collected and analyzed the literature this paper needed. All authors have read and agreed to the published version of the manuscript

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ikushima, T.; Aritomi, H.; Morisita, J. Radioadaptiveresponse; Efficient repair of radiation induced DNA damage in adapted cell. *Mutat. Res.* **1996**, *358*, 193–198. [CrossRef]
2. Cho, K.; Jwa, M.-S.; Moon, H.-N.; Hur, S.-P.; Kim, D.; Yeo, I.-K. Hormetic effect of ^{60}Co gamma radiation on tolerance to salinity and temperature stress in *Haliotis discus discus*. *Aquaculture* **2016**, *451*, 473–479. [CrossRef]
3. Mattson, M.P. Awareness of Hormesis Will Enhance Future Research in Basic and Applied Neuroscience. *Crit. Rev. Toxicol.* **2008**, *38*, 633–639. [CrossRef] [PubMed]
4. Lee, H.Y.; Kim, J.S.; Baek, M.H.; Lee, Y.K.; Im, D.S. Effects of low dose γ-radiation on the growth, activities of enzymes and photosynthetic activities of gourd (*Lagenaria siceraria*). *Korean J. Environ. Biol.* **2002**, *20*, 197–204.
5. Kim, K.J.; Choe, M.K.; Yeo, I.K. Effect of gamma-ray on growth of juvenile abalone *Haliotis discus hannai*. *Korean J. Environ. Biol.* **2007**, *25*, 107–114.
6. Jwa, M.S.; Yeo, I.K. Early growth changes in juvenile abalone *Haliotis discus discus* produced by irradiation with low-dose 60co gamma-rays. *Korean J. Fish. Aquat. Sci.* **2015**, *48*, 193–199. [CrossRef]
7. Day, R.W.; Fleming, A.E. The determinants and measurement of abalone growth. In *Abalone of the World: Biology, Fisheries and Culture*; Shepherd, S.A., Tegner, M.J., Guzman, S.A., Del Proo, S.A., Eds.; Fishing News Books; Blackwell: Oxford, UK, 1992; pp. 141–168.
8. Huchette, M.H.S.; Koh, C.S.; Day, R.W. Growth of juvenile blacklip abalone (*Haliotis rubra*) in aquaculture tank: Effects of density and ammonia. *Aquaculture* **2003**, *219*, 457–470. [CrossRef]
9. Kim, T.H.; Yang, M.H.; Choe, M.K.; Han, S.J.; Yeo, I.K. Physiological studies on acute water-temperature stress of juvenile abalone, *Haliotis discus hannai*. *Korean J. Aquacult.* **2005**, *18*, 7–12.
10. Jwa, M.S.; Kang, K.P.; Choe, M.K.; Yeo, I.K. Effects of low salinity stresses on the physiology of disc abalone, *Haliotis discus discus*. *J. Fish Pathol.* **2009**, *22*, 293–303.
11. Choe, M.K.; Yang, S.G.; Won, S.H.; Park, C.J.; Han, S.J.; Yeo, I.K. Estimation of genetic parameters for growth-related traits in 9-month old of two Korean abalone subspecies, *Haliotis discus hannai* and *H. discus discus*, by using multiple traits of animal model. *Korean J. Fish. Aquat. Sci.* **2009**, *42*, 591–599.
12. Kim, J.K.; Lim, S.H.; Kim, I.J.; Lee, Y.H.; Kang, H.W. Mutagenesis of *Pleurotus eryngii* by gamma-ray irradiation. *Korean J. Mycol.* **2012**, *40*, 93–97. [CrossRef]
13. Kim, J.B.; Lee, K.J.; Kim, D.S.; Ha, B.K.; Kim, S.H.; Song, H.S.; Kang, S.Y. An improved soybean cultivar 'Wonyul' with resistance to phomopsis seed decay by mutation breeding. *Korean J. Breed Sci.* **2013**, *45*, 293–297. [CrossRef]
14. Dubner, D.; Gisone, P.; Jaitovich, I.; Perez, M. Free radicals production and estimation of oxidative stress related to γ irradiation. *Biol. Trace Element Res.* **1995**, *47*, 265–270. [CrossRef] [PubMed]
15. Kim, Y.S.; Woo, C.K.; Lee, Y.S.; Koh, J.K.; Chun, H.C.; Lee, M.Z. Expression of *Jun* and *p53* genes from the brain of rats irradiated with 60Co γ-ray. *J. Korean Soc. Radiol.* **1996**, *14*, 265–280.
16. Lee, E.K.; Kim, J.S.; Lee, Y.K.; Lee, Y.B. Effects of low dose γ-ray irradiation on the germination and growth in red pepper. *J. Kor Soc. Hort. Sci.* **1998**, *39*, 670–675.
17. Kim, J.S.; Lee, E.K.; Back, M.H.; Kim, D.H.; Lee, Y.B. Influence of low dose γ radiation on the physiology of germinative seed of vegetable crops. *Korean J. Environ. Agric.* **2000**, *19*, 58–61.
18. Kim, J.S.; Lee, Y.K.; Park, H.S.; Back, M.H.; Chung, K.H. Effects of low dose gamma radiation on the early growth and physiological activity of gourd (*Lagenaria leucanthal*). *Korean J. Environ. Agric.* **2000**, *19*, 142–146.
19. Kim, J.K.; Lee, C.J.; Lee, Y.K.; Song, K.W.; Yoon, Y.D. Cell cycle evaluation of granulosa cells in the γ-radiated mouse ovarian follicles. *J. Radiat. Prot.* **1999**, *24*, 17–22.
20. Koeep, R.; Kramer, M. Photosynthetic activity and distribution of photoassimilated ^{14}C in seedlings of zeamays grown from gama-irradiated seeds. *Photosynthetica* **1981**, *15*, 484–493.
21. Kim, Y.S.; Ji, S.C.; Biswajit, K.B.; Amal, B.; Jeong, G.S.; Osamu, M.; Kenji, T. Growth and environmental tolerances (Water Temperature and Low Salinity) of hybrid female red sea bream *Pagrus major* × male black sea bream *Acanthopagrus schlegelii*. *Korean J. Fish. Aquat. Sci.* **2011**, *44*, 276–283. [CrossRef]

22. Wang, W.; Mai, K.; Zhang, W.; Xu, W.; Ai, Q.; Liufu, Z.; Li, H. Dietary selenium requirement and its toxicity in juvenile abalone *Haliotis discus hannai* Ino. *Aquaculture* **2012**, *333*, 42–46. [CrossRef]
23. Mohandas, S. Effect of presowing seed treatment with molybdenum and cobalt on growth, nitrogen and yield in bean (*Phaseolus vulgaris* L.). *Plant Soil* **1985**, *86*, 283–285. [CrossRef]