



## LD50 OF GAMMA RAY IRRADIATION EMPLOYED IN SULAWESI MEDAKA *Oryzias woworae*

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# LD<sub>50</sub> OF GAMMA RAY IRRADIATION EMPLOYED IN SULAWESI MEDAKA *Oryzias woworae*

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Key words: gamma ray irradiation, medaka, median lethal dose, gender.

## ABSTRACT

This experiment aims to estimate the median lethal doses (LD<sub>50</sub>) of irradiation (IR) for the matured freshwater oviparous ornamental fish, Sulawesi medaka (*Oryzias woworae*). Both male and female were treated with 0, 10, 20, 25, 30, 35, 40, 60, 200, 400, 600 and 800 Gy. Each treatment was conducted in triplicate. The 96, 168 and 336 h- LD<sub>50</sub> of male and female were 643.1, 47.0, 33.8 and 646.8, 42.5, 35.7 Gy, respectively.

## I. INTRODUCTION

The demands of ornamental fish as a commodity in the global market expressing a strong preference for novel and unique species are being met by artificial selection and breeding of multiple generations (Bruckner, 2005; Moreau and Coomes, 2007). Both wild caught and captive bred medaka (*Oryzias* spp.) have served as popular aquarium strains and fluorescent medaka have dominated the market in recent years, especially as the fluorescent ones are the model for genetic modification organisms (Wittbrodt et al., 2002; Furutani-Seiki et al., 2004). The aquatic animals, including ornamental fish, have not been protected by patent and the mass production of ornamental fish is mostly made by the fish farmers who possess the ability to propagate (Ng and Tan, 1997). Therefore, this not only jeopardizes ornamental fish industry development but also decreases the profit of originators (Gong, 2001).

Gamma ray irradiation (IR) causes sterility by damaging the DNA and thus leads to abnormal mitosis or meiosis (Lee, 2000; Coates et al., 2004; Snyder and Morgan, 2004), but it does no harm to the appearance of fish. Sterilization can prevent the parents from having offspring with reproductive capability, and significantly reduce the impact of alien invasion

on the ecological environment (Hanson, 1990; Sellars et al., 2007). IR has been successfully used as an effective sterility technique for insects (Dominiak et al., 2003) and such aquatic species as rainbow trout *Oncorhynchus mykiss* (Konno and Tashiro, 1982), Atlantic salmon *Salmo solar* (Thorpe et al., 1987), male marine lamprey *Petromyzon marinus* (Hanson, 1990) and oysters *Crassostrea virginica* (Ardjosoediro, 2001). Such practice can preserve or maintain native species, and avoid genetic contamination (Sellars et al., 2005; 2007).

Although IR treatment has successfully sterilized many plants and animals, the effects of such treatment on freshwater ornamental oviparous fish remain unknown. Therefore, the objective of this experiment was to establish a safe dose of IR used in ornamental fish, Sulawesi medaka.

## II. MATERIALS AND METHODS

The captive bred Sulawesi medaka, *Oryzias woworae*, (male and female were  $0.79 \pm 0.10$  and  $0.82 \pm 0.20$  g, respectively) were purchased from the fish farm in Pingtung and immediately sent to National Taiwan Ocean University, Keelung, Taiwan. Two thousand fish were acclimated in a 200 L tank equipped with an air-lifting filter bed in which pH, temperature, and dissolved oxygen were kept at  $7.0 \pm 0.2$ ,  $26.0 \pm 1.0$  °C and  $6.0 \pm 0.2$  mg l<sup>-1</sup>, respectively. Twenty percent of tank water was replaced per day with dechlorinated and aerated water. The concentrations of total ammonia-nitrogen (TAN) and nitrite-nitrogen (NO<sub>2</sub>-N) remained 0.2 mg l<sup>-1</sup> and 50 µg l<sup>-1</sup>, respectively. The fish were fed with commercial feed (Tropical Micro Pellets, Hikari, Japan) of 2% of total body weight twice daily. Feeding was discontinued 48 hrs prior to irradiation.

The fish were not separated until matured medaka could be distinguished by the color, the margins of tail and anal fin as well as the sexual dimorphism. Fish were divided into two groups by gender. Twenty gender-specific fish were placed in a polyethylene bag (295 × 120 mm) filled with 500 ml water and the same volume of oxygen, and then the bag was sealed with a rubber band. There were totally seventy eight bags, which were put in a dark closed environment, a Styrofoam box with water temperature ( $26.0 \pm 1.0$  °C) before irradiation.

The irradiation was conducted 5 M underwater at the Atomic Energy Council, Taoyuan, Taiwan, and the gamma-ray

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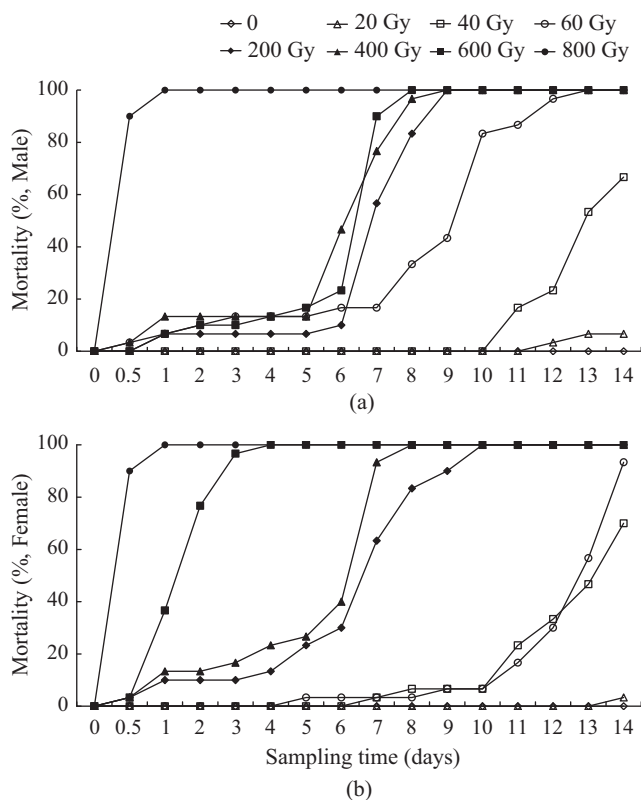


Fig. 1. The accumulated mortality of matured male medaka (a) and female (b) treated with 0, 20, 40, 60, 200, 400, 600 and 800 Gy and cultured for 14 days after irradiation.

source was produced by cobalt-60 ( $^{60}\text{Co}$ ). The dosage was a translation of the radiation exposure time (5 Gray/min). The bags were placed in a metal chamber (30 × 12 × 25 cm), and the treatments were as follows: 0 (control), 10, 20, 25, 30, 35, 40, 60, 200, 300, 400, 600 and 800 Gy. Each treatment was in triplicate.

Soon after the irradiation, the fish were brought back to National Taiwan Ocean University for further study. The fish of each bag was moved to a 1L acryl tank and placed in a circulation system with constant temperature ( $26.0 \pm 0.2$  °C), light controlled (12 L:12D), 0.05 mg l<sup>-1</sup> TAN and 0.1 mg l<sup>-1</sup> NO<sub>2</sub>-N. There was no feeding during experiment period. The survival of each treatment was examined and recorded until 14 days (336 hrs). The dead fish were removed and the number of death was recorded per day. In this experiment, the median lethal dose (LD<sub>50</sub>) of IR is the dose required to kill half the members of a treated organisms for certain duration after irradiation. The LD<sub>50</sub> was calculated by computer program (GW Basic 3.23).

LD<sub>50</sub> was calculated by computer formula LD<sub>50</sub> GB Basic 3.23. Linear regression was used to determine the relationship between anesthetics concentration and induction to anesthesia time. One-way analyses of variance (ANOVA) were used to determine significant ( $P < 0.05$ ) of different sex of fish treated with IR and the mortality of different time elapsed after IR.

Table 1. The median lethal dosage (95% confidence limits) of gamma ray irradiation for different gender of Sulawesi medaka *Oryzias woworae* after different time elapsed.

Gender	Time after gamma ray irradiated			
	24 hrs	96 hrs	168 hrs	336 hrs
Female	549.12 Gy (412-732 Gy)	643.14 Gy (60-6862 Gy) <sup>b</sup>	46.99 Gy (15-148Gy) <sup>a</sup>	33.75 Gy (27-43 Gy) <sup>b</sup>
Male	-	646.83 Gy (158-2637 Gy) <sup>a</sup>	42.51 Gy (11-165Gy) <sup>b</sup>	35.68 Gy (26-48 Gy) <sup>a</sup>

<sup>a,b</sup> Values in each column having different superscripts are significantly different ( $P < 0.05$ ).

-: not detected.

### III. RESULTS AND DISCUSSION

The accumulated mortalities of matured male and female treated with 0, 20, 40, 60, 200, 400, 600 and 800 Gy, respectively, for 14 days were shown in Fig. 1. Both matured male and female treated with 800 Gy showed immediate death (less than one day). The matured male treated with 600 Gy reached 100% mortality after seven days while the matured female treated with the same dosage died after three days. However, the mortality of matured male treated with low dosage (60 Gy) dramatically increased after 7 days and the mortality of matured female treated with the same low dosage dramatically increased after 12 days. We found that male fish treated with low dosage IR had seriously fought. Therefore, the fight of male fish treated with 60 Gy after 7 days (168 hrs) caused fish to have dramatically increased mortality. It showed that when treated with above 600 Gy, female were more susceptible than male. The male fish treated with low dosage IR (below 60 Gy) was more susceptible than female.

The 96-, 168- and 336 h- LD<sub>50</sub> of IR were shown in Table 1. The LD<sub>50</sub> calculated from the average accumulated mortality of the matured female and male at 96, 168 and 336 hrs were 643.1, 47.0, 33.8 and 646.8, 42.5, 35.7 Gy, respectively. The 96- and 336 h- LD<sub>50</sub> of IR for male were significantly higher than those for female. Due to the dramatically increased mortality of male fish treated with 60 Gy, 168 h- LD<sub>50</sub> for male was lower than that for female.

In aquatic radiobiology, Anderson and Harrison (1986) indicated that lethal levels of chronic and acute regime of fish ranged from 3.75-550 Gy. Lee (2000) found that adult female freshwater prawn (*Macrobrachium rosenbergii*) showed higher IR sensitivity than adult male when they were treated with 600 Gy and Sellars et al. (2005; 2007) investigated the sterility induction of different gender of kuruma shrimp (*Marsupenaeus japonicus*) treated with IR and indicated that the dose sensitivity was affected by the gender. The result of the present experiment is similar to that of the previous studies obtained. It can be inferred that the female aquatic animals have higher susceptibility than the male aquatic animals.

The female grass shrimp, *Palaemonetes pugio* treated with 9.75 Gy failed to produce eggs (Rees, 1962), and male and

female freshwater giant prawn are sterilized by exposing to 10 and 15 Gy, respectively (Lee, 2000). Male and female medaka treated with 10.4 and 3 Gy, respectively can increase mutation frequencies of their loci (Tsyusko et al., 2007). Jarvis and Knowles (2003) treated zebrafish with gamma rays and found the DNA lesions of zebrafish 24 hrs after treatment. However, the study of mortality of aquatic organisms treated with irradiation was scarce. Hillary (2013) indicated that zebrafish embryos treated with 15 Gy and 30 Gy had 75% and 80% mortality, respectively. The LD<sub>50</sub> of IR for Sulawesi medaka established in this study provides a guideline for the future IR application in ornamental industry.

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