



Effect of cobalt-60 gamma radiation on reproductive disturbance in freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879)

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ABSTRACT

The present study was designed to evaluate the effect of acute level of ⁶⁰Co gamma radiation on fecundity of freshwater prawn *Macrobrachium rosenbergii*. Prawns were exposed to four different dose levels (3, 30, 300 and 3000 mGy) and their reproductive disturbances i.e Gonadosomatic Index (GSI), Egg Clutch somatic Index (ESI), Egg Counts and Egg hatching rates were calculated. The experimental group showed significant reduction in GSI (0.47 ± 0.01) & ESI (1.22 ± 0.08) after exposure to ⁶⁰Co gamma radiation. Egg Count (3713 ± 21) and Egg hatching rates (3798 ± 11) were significantly reduced in all irradiated groups. The number of dead larva increased with the increasing level of doses. With the increase in dosage level, the gonad and egg clutch weight were decreased which likely lead to reduced number of eggs. Our results proved that even low level of ionizing radiation (⁶⁰Co) affects the fecundities of freshwater crustacean *M. rosenbergii*.

1. Introduction

Nowadays, various international directives (ERICA and PROTECT) show interest on studying the impact of radioactive emissions on the environment, particularly the ionizing radiation impact on non-human biota of both aquatic and terrestrial organisms, to develop the protective measures against the radioactive pollution [1,2]. The emergence of ionizing radiation into the environment is by two major sources such as natural and anthropogenic. Nuclear weapons testing, nuclear disasters and permitted discharges from nuclear reprocessing plants are the three major sources of anthropogenic radionuclides in the environment [3]. Releasing of radioactive waste from nuclear facilities [4] either accidentally or intentionally makes the aquatic ecosystem as a sink for the radionuclides [5]. However, a very few attempt has only been made in this aspect [6–16], (Tables 1 and 2). Reproduction, the most sensitive endpoint of radiation exposure in non-human biota [17], is considered as the eco-toxicological and environmental risk assessment studies [18,19]. Hence, an attempt was made to analyze the impact of cobalt 60 gamma (ionizing) radiation on fresh water crustaceans *Macrobrachium rosenbergii* egg production and its further development in low dose level (mGy). *M. rosenbergii*, a native prawn species of Southeast

Asian countries [20], constitutes a major species of fishery in River Cauvery (Tamil Nadu, India), an important perennial river of South India [21].

2. Materials and methods

2.1. Experimental prawn & irradiation

Live prawns were purchased from the Dhanalakshmi Prawn Farm, Cuddalore, Tamil Nadu (India) and acclimatized under controlled condition using indoor fiber water tanks (1.5 × 1.0 m) with proper aeration. The setup was maintained in the Environmental Research Laboratory, Jamal Mohamed College, Tiruchirappalli (Tamil Nadu, India) and maintained in a 12:12 h (light/dark) photoperiod. Prawns were fed with boiled and chopped goat liver *ad libitum* every day.

Pre-moulted females and mature male prawns were irradiated using Theratron phoenix (P-33) tele cobalt unit (Canada) having specification in the dose rate of 360 mGy/min in a ⁶⁰Co radionuclide source located in GVN Cancer Cure Research Center and Hospital, Tiruchirappalli, Tamilnadu, India. The experimental animals were placed in the polypropylene boxes (0.25 × 0.05 m (L × B × H) of capacity 1.5 L water).

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Table 1List of Gamma Radiation (^{60}Co) studies in non-human biota by various researchers (www.fredrica-online.org; IAEA, 2002).

Species (Common name)	Dose Rate (Gy)	Radiation Effect	References
Pisces			
<i>Esox lucius</i> L. (Pike)	0, 2	Reproduction (delayed hatching), anomaly	[6]
<i>Cyprinus carpio</i> (Carp)	5, 7.5, 10, 15, 20, 40, 80, 120, 160	Reproduction	[7]
<i>Oncorhynchus tshawytscha</i> (Chinook salmon)	0, 0.41, 0.95, 2.3, 3.5, 8.1, 17, 42	Reproduction (retardation of Gonadal differentiation)	[8]
<i>Salmo gairdnerii</i> (Rainbow trout)	0.25, 0.5, 1, 2, 4	Reproduction (embryo abnormalities)	[9]
<i>Tinca tinca</i> L. (Tench)	0, 0.04, 0.25, 0.5, 1, 2.5, 40.25, 40.5, 41, 42.5	Reproduction (reduced survival rate in larvae).	[10]
Crustaceans			
<i>Physa heterostopa</i> (Aquatic Snail)	1, 10, 25	Reproduction and fecundity	[11]
<i>Mercenaria mercenaria</i> (Clams)	0.0, 0.25, 2.5, 28, 1020	Growth and Survival monitored	[12]
<i>Mytilus edulis</i> (Mussel)	0.9 or 2.0 μ Gy	Observation of Cilia beating monitored and behavioural response also	[13]
<i>Biomphalarica glabrata</i> (Snail)	0, 2.5, 10, 20	Counted off spring	[14]

Table 2

Rationale for establishing a derived consideration reference level (DCRL) for a hypothetical reference animal or plant.

Source: Table 2 from ICRP 108 publication (ICRP, 2008b).

Dose rate interval (mGy/ day)	Observations (hypothetical)	Concern
1000–10,000	Significant mortality	High to very high
100–1000	Population disturbance from prolonged exposure	High if prolonged exposure
10–100	Effects on morbidity	Increasing
1–10	Some reproductive disturbance	DCRL band
0.1–1	No effects observed	Very low to low
0.01–0.1	Close to or within natural background, no observation of effects	Nil to very low

The experiment consisted of five groups of six prawns each (Three replicates *i.e.* $n = 18$). Lethal dosage of ^{60}Co gamma radiation for *M. rosenbergii* was first studied and analyzed by SPSS tool - probit analysis. One group was considered as the control and the other four groups were exposed to 3, 30, 300 and 3000 mGy each and were observed for the next 96 h.

2.2. Morphometric assessment

The individual weight of each prawn was measured in grams (gm) using a digital balance (DENVER). The sex of each specimen was determined by the visual observation of the base of the fifth pair of pereopods [22]. The different stages of ovarian development were classified based on the color, size and outline of the ovary [23].

2.3. Assessment of organo somatic index

At the end of the experiment, the weight of each prawn was measured. The prawns (2 pairs in each group) were randomly selected and cut open in the mid-dorsal line and their adjoining tissues were removed [24]. The gonads of both control and irradiated group of prawns were collected and weighed for Gonado Somatic Index [25,26]. The egg clutches were removed for the Egg clutch somatic index [27].

Calculation of GSI and ESI:

$$\text{Gonado somatic index} = \frac{\text{Gonad weight (gm)}}{\text{Total body weight (gm)}} \times 100$$

$$\text{Egg clutch somatic index} = \frac{\text{Egg clutch weight (gm)}}{\text{Total body weight (gm)}} \times 100$$

2.4. Egg counting & hatching rate

Egg clusters were removed 7 days after spawning from both control and the irradiated broods. Eggs were carefully removed from the brood pouch by following the standard procedures [28]. Eggs were counted manually by using the magnifying glass and observed under the stereomicroscope [29]. The eggs were incubated *in vitro* in a fiber glass tank at a salinity of 8ppt with moderate aeration. The hatching rate was calculated from the number of eggs in a brood from the number of larvae that hatched out. The number of live larvae and that of the dead eggs were observed after 24 h after hatching [30,27].

2.5. Statistical analyses

The obtained values were expressed as the mean \pm standard deviation (SD). Differences between groups were assessed by one-way analysis of variance using the SPSS software package for windows (version 16.0). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Lethal dose

Lethal dose of *M. rosenbergii* was determined using the probit analysis and LD_{50} of gamma irradiated *M. rosenbergii* was identified at 30 Gy (Fig. 1).

3.2. GSI and ESI

Yellowish or bright orange coloured eggs were observed in the mature females where the brood pouch was located in the cephalothorax, which was visible through the carapace. The gonado somatic index of the control group (Table 3) was 0.59 ± 0.02 . A radiation stress which developed in prawns, reduced the GSI in all the irradiated groups to 0.51 ± 0.01 , 0.52 ± 0.03 , 0.5 ± 0.01 and 0.47 ± 0.01 respectively (Fig. 2).

The collected egg clutches were placed in a filter paper to avoid moisture content for few seconds, and then it was weighed and the values were entered. The obtained value for the control group was 2.11 ± 0.14 (Table 3). The ESI range had significantly decreased to 1.84 ± 0.01 , 1.66 ± 0.06 , 1.21 ± 0.05 and 1.22 ± 0.08 in 3, 30, 300, 3000 mGy respectively (Fig. 2).

3.3. Egg counting rate and hatching rate

The collected eggs were observed using a stereomicroscope along with water (8ppt). The number of eggs in all three replicates, and after hatching the live post larvae's (PL) were counted and recorded

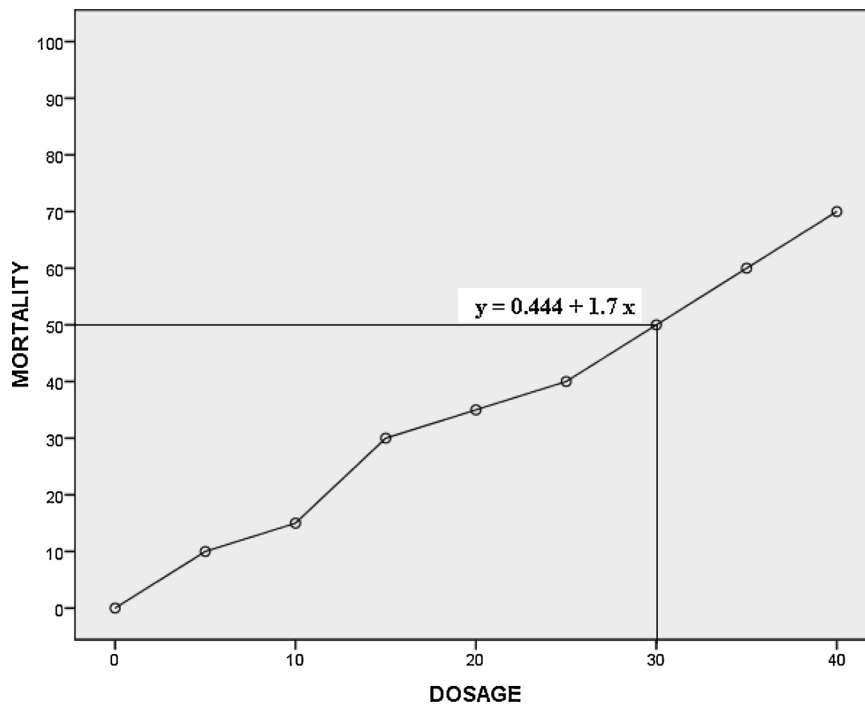


Fig. 1. Probit analysis Graph showing LD₅₀ in *M. rosenbergii*.

Table 3

Gonado somatic and Egg clutch Somatic indices of *Macrobrachium rosenbergii* exposed with ⁶⁰Co gamma radiation (n = 18).

Dose level (mGy)	Mean ± SD	
	GSI	ESI
Control	0.59 ± 0.02	2.11 ± 0.14
3	0.51 ± 0.01	1.84 ± 0.01
30	0.52 ± 0.03	1.66 ± 0.06
300	0.50 ± 0.01	1.21 ± 0.05
3000	0.47 ± 0.01	1.22 ± 0.08

Table 4

Total egg count and hatched egg count in control and ⁶⁰Co gamma irradiated groups *M. rosenbergii* (n = 18).

Dose (mGy)	No. of. Egg Count (Mean ± SD)	No. of. Egg Hatched (Mean ± SD)
Control	4850 ± 23	3798 ± 11
3	4739 ± 15	3672 ± 32
30	4511 ± 35	3503 ± 12
300	4030 ± 56	3005 ± 40
3000	3713 ± 21	2813 ± 25

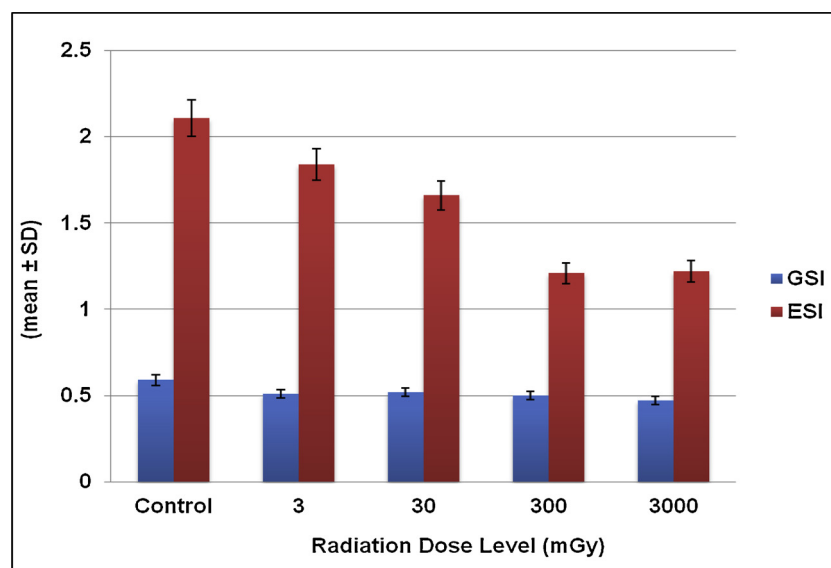


Fig. 2. Gonado somatic and Egg clutch somatic indices of *M. rosenbergii* exposed with ⁶⁰Co gamma radiation.

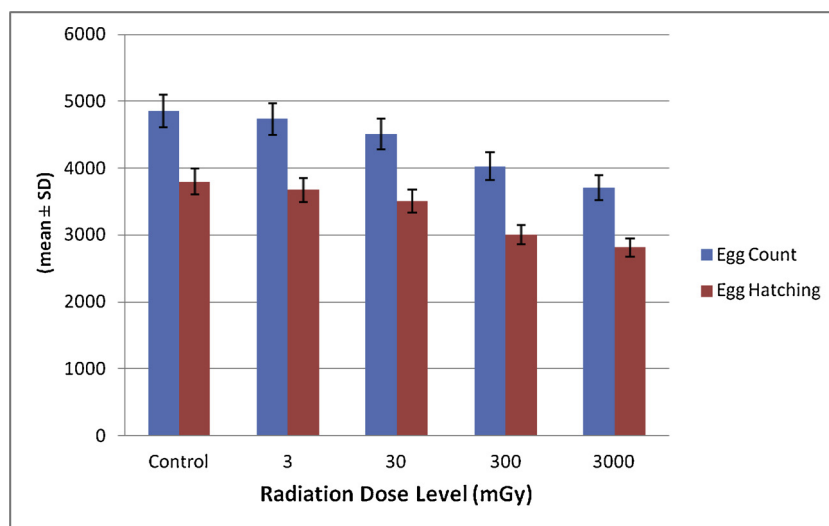


Fig. 3. Egg counting and hatching rate of control and ^{60}Co gamma irradiated prawn *M. rosenbergii*.

(Table 4). The dead larvae were removed from the tank. In the control group, 4850 ± 23 eggs were observed. Irradiated groups showed decreased number of eggs when compared to that of the control. The least number of eggs were found in 3000 mGy of the irradiated group as of 3713 ± 21 eggs (Fig. 3). In the control group, 3798 ± 11 juveniles were counted. The number of dead ones had significantly increased with increased doses. In 3000 mGy, 2813 ± 25 PL was counted (Fig. 3).

4. Discussion

In the crustaceans, the female reproductive outputs were considered as the typical endpoints including the production of new eggs [31] as well as the hatchability of eggs [32,33]. Organosomatic indices reflected the status of organ systems and their changes by various environmental factors and stressors [34]. Reduced gonado somatic index was observed in irradiated groups due to the stress which was developed by the ^{60}Co gamma ionizing radiation.

[35] estimated the normal fecundity of *M. rosenbergii* by counting the number of eggs on the pleopods where it ranged from 20,000 to 70,000 eggs and their mean number of eggs per female was around 4500 eggs which was similar to our control results. Infrared (IR) radiation treated female *M. rosenbergii* (0, 5, 10, 15 Gy) and *P. japonicus* (20 Gy) showed 100% and 52.2% (relative to non-irradiated females) reduced fecundity [36].

The reduced fecundity observed in the crustacean *P. japonicus* was due to the deleterious effect of ionizing radiation [37,38]. A significantly reduced fecundity was observed in the female brine shrimp (*Artemia* sp.) exposed to IR (1–5 Gy) and the absence of oocytes production after the exposure to 10 Gy (Squire, 1970). Larvae Hatch Fecundity (LHF) is the number of larvae released from the egg mass following the incubation [39], which was reduced in the irradiated prawn than that of the control.

Several recent reports and international bodies were grappling with the problems of regulating exposure of biota [40–44] and the most fundamental issue was due to the lack of adequate scientific data with concern to low dose exposure effects.

Different organizations such as USDOE, NCRP, IAEA, ICRP and UNSCEAR had published many data about the benchmark values regarding to the environmental radiation protection in various countries [45]. The FREDERICA database contains over 30,000 data entries from a number of international radiation effects whereas the directives contains data on chronic dose ranges of $0 - > 10,000 \mu\text{Gy/hr}$ [46].

There is a lacuna in acute ionizing radiation studies in freshwater

crustacean species. Hence, this work and its related other studies [47,48] provides the necessary information about the biological effect of ionizing radiation to develop the permissible dose levels. Similar kind of acute radiation studies were performed in fresh water *O. mossambicus* [49] by the same team, hence we suggested having in other freshwater species also.

5. Conclusion

The study proved that even the minimal dose of 3 mGy of ^{60}Co gamma irradiation is sufficient to reduce the egg production, growth and hatching rate of *M. rosenbergii*. Hence, it is suggested that the outputs from the nuclear industries, hospitals and research institutes should be kept below 3 mGy of ^{60}Co gamma irradiation.

Declaration of Competing Interest

The authors declare no conflict of interest.

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