

1 **Gamma irradiation of resting eggs of *Moina macrocopa* affects individual and population**  
2 **performance of hatchlings**

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36

37 **Abstract**

38 We investigated the effects of  $\gamma$ -radiation on the survival of resting eggs of the cladoceran  
39 *Moina macrocopa*, on the parameters of the life cycle of neonates hatched from the irradiated  
40 eggs and on the performance of the population initiated from irradiated eggs. The study showed  
41 that  $\gamma$ -radiation in a range of doses from the background level to 100 Gy had no effect on  
42 survival of irradiated eggs. The absorbed dose of 200 Gy was lethal to resting eggs of *M.*  
43 *macrocopa*. The number of clutches and net reproductive rate ( $R_0$ ) of hatchlings from eggs  
44 exposed to radiation were the strongly affected parameters. The number of clutches was  
45 drastically reduced for females hatched from egg exposed to 80-100 Gy. The most sensitive  
46 parameter was the  $R_0$ . The estimated  $ED_{50}$  for the  $R_0$  (effective dose that induces 50%  $R_0$   
47 reduction) was 50 Gy. Population performance was also affected by the irradiation of the  
48 resting stage of animals that initiated population. Populations that was initiated from hatchlings  
49 from resting eggs exposed to 100 Gy was of smaller size and with fewer juvenile and  
50 parthenogenetic females in comparison with control populations. Thus, we determined the  
51 dose-response relationship for the effect of gamma radiation on survival of resting eggs and  
52 individual and population responses of hatchlings from irradiated resting eggs. We conclude  
53 that for highly polluted areas contamination of bottom sediments with radioactive materials  
54 could affect zooplankton communities through adverse chronic effects on resting eggs, which  
55 will be transmitted to hatchlings at individual or population levels.

56

57 **Key words:**  $\gamma$ -radiation, Cladocera, resting eggs, life cycle parameters, population performance

58

## 59 1. Introduction

60 Background radioactivity is a natural phenomenon, but over the past century, its level  
61 has increased globally and locally due to various anthropogenic activities such as nuclear  
62 weapon tests, nuclear accidents and routine operation of nuclear power industries (United  
63 Nations Scientific Committee on the Effects of Atomic Radiation, 2008). Artificial  
64 radionuclides enter aquatic ecosystems due to discharges from nuclear-power facilities,  
65 washouts from water catchment areas and nuclear fall-outs (Van der Stricht and Janssens,  
66 2010). Bottom sediments serve as a sink for various anthropogenic pollutants including  
67 radionuclides (e.g. Kansanen et al., 1991), causing benthic biota (animals and plants) to be  
68 exposed to anthropogenic radionuclides.

69 Cladocerans are widespread species that dominate zooplankton in various aquatic  
70 ecosystems. These filter feeders play a vital role in aquatic food webs, as they transfer organic  
71 carbon from primary producers to higher food levels such as fish or invertebrate predators.  
72 Under favorable conditions, they reproduce by parthenogenesis, which allows them to increase  
73 in numbers quickly and control the lower food web level (Lampert et al., 1986). Due to their  
74 importance for food webs, ease of handling, sensitivity to various factors and fast reproduction  
75 rates, some typical species of Cladocera, such as *Daphnia* or *Moina*, are often used as model  
76 organisms in toxicological and biosafety research (Guilhermino et al., 2000).

77 There are numerous experimental data on the effect of ionizing radiation on the life  
78 cycle and population characteristics of Cladocera (e.g. Alonzo et al., 2008; Massarina et al.,  
79 2010; Sarapultseva and Gorski, 2013). Recent studies have also demonstrated the  
80 transgenerational effects of parental exposure to ionizing radiation on survival and fertility of  
81 directly exposed females of *Daphnia magna* and their offspring (Sarapultseva and Dubrova,  
82 2016). However, in natural habitats, cladocerans are not always present in active stage. Usually,  
83 under adverse conditions, many species of Cladocera produce resting eggs (Alekseev, 2007).  
84 This is the strategy to survive either seasonal or occasional unfavorable conditions. Resting  
85 eggs are able to survive drying and freezing (Radzikowski, 2013). Resting eggs form an egg  
86 bank at the bottom of a water body. This bank is a source of genetic diversity and usually  
87 replenishes the population after periods of population decline (Brendonck, 2003). Cyclic  
88 reproduction of Cladocera follows the seasonal pattern. In spring, the active zooplankton are  
89 recruited from the bank of resting eggs. Under favorable conditions zooplankton quickly  
90 increase in numbers by parthenogenetic reproduction. In autumn, population declines in  
91 numbers and produces resting eggs, which will overwinter at the bottom to hatch next spring.

92 Despite the obvious importance of resting eggs for the ecosystem development, there  
93 remains a paucity of evidence on the effect of ionizing radiation on resting eggs of Cladocera

94 and subsequent development of hatchlings from irradiated eggs. Recently we presented the data  
95 that demonstrated the effect of gamma radiation on the hatching success of resting eggs of  
96 cladoceran *Moina macrocopa* and some life cycle parameters of hatchlings from irradiated eggs  
97 (Zadereev et al., 2016). However, in that study, we did not determine the lethal dose that  
98 inhibits hatching of resting eggs. We did not determine population consequences of irradiation  
99 of resting eggs either. In natural habitats, contaminated bottom sediments may affect the  
100 survival of resting eggs. Also, hatchlings from irradiated resting eggs may demonstrate poor  
101 performance, which may influence population dynamics. Thus, to understand ecological effects  
102 of the sediments contaminated by anthropogenic radionuclides, it is important to study the  
103 effect of ionizing radiation both on the survival of resting eggs and on the performance of  
104 animals hatched from irradiated eggs.

105 In this study, resting eggs of a typical cladoceran, *Moina macrocopa*, were exposed to  
106 the wide range of doses of gamma radiation in order to: 1) determine the dose that prevents  
107 resting eggs from hatching (lethal dose), 2) follow the hatchlings from irradiated resting eggs in  
108 life table experiments to determine the response of life cycle parameters of active animals to  
109 the irradiation of the resting stage, and 3) initiate populations from irradiated resting eggs to  
110 compare the performance of populations emerging from the resting eggs that absorbed different  
111 doses of radiation.

112

## 113 **2. Material and methods**

### 114 *2.1. Experimental conditions*

115 In our experiments, we used culture of cladoceran *Moina macrocopa* that had been  
116 maintained in the laboratory of the Institute of Biophysics SB RAS (Krasnoyarsk) for the last  
117 ten years. The culture was initiated from resting eggs from a small pond near the Rybinsk  
118 reservoir (Western part of Russia, the Volga River basin), kindly collected and provided by  
119 Vladimir Chugunov. All experiments were performed in a climate chamber with the  
120 temperature optimal for growth and reproduction of *M. macrocopa* ( $25\pm 1^\circ\text{C}$ ) and photoperiod  
121 (16 h light–8 h dark) (Zadereev and Gubanov, 1996; Oh and Choi, 2012). Aged, for at least 24  
122 hours, and aerated tap water was used as a culture medium. The animals were fed with the  
123 unicellular green alga *Chlorella vulgaris*, which was grown in batch culture in 500 mL flasks in  
124 Tamiya medium. Before being used as food, the algae were concentrated by centrifugation  
125 (1200 g). The concentrated algae that were used as food in experiments were kept in the  
126 refrigerator for no longer than two weeks. These experimental conditions were tested in our  
127 numerous previous experiments and proved to be optimal for *M. macrocopa* growth and  
128 parthenogenetic reproduction, quality of controls can be checked based on previously published

129 experiments (Zadereev and Gubanov, 1996; Zadereev, Gubanov, Egorov, 1997). Concentration  
130 of the algae in the medium was adjusted to a desired level by dilution and determined with a  
131 CASY TTC particle counter (SCHÄRFE SYSTEM GmbH, Germany).

## 132 2.2. Irradiation of resting eggs

133 Resting eggs for experiments were obtained from the batch culture of *M. macrocopa*. The  
134 batch culture was initiated from ca. 40-50 resting eggs and cultivated in 4 L of the medium  
135 renewed every 3 days, with the concentration of *Chlorella* adjusted to 1 million cells per mL.  
136 Under these experimental conditions, after hatching from resting eggs, population increased in  
137 numbers quickly and a large number of resting eggs (several thousands) were produced in a  
138 short period of two-three weeks. The resting eggs were routinely collected from the culture,  
139 checked for the fullness (fertilized ephippium of *M. macrocopa* contains two resting eggs) and  
140 were stored in the dark at 4°C.

141 We studied the sensitivity of resting eggs to ionizing radiation in the dose range varied  
142 from the background level to 200 Gy from two point sources of  $^{137}\text{Cs}$  (activities  $12.4 \cdot 10^6$  and  
143  $1.12 \cdot 10^{10}$  Bq respectively) and an industrial high-frequency electron accelerator. For each  
144 irradiation experiment, we selected undamaged ephippia containing two fertilized resting eggs  
145 of *M. macrocopa*. For each absorbed dose, 30–50 ephippia were irradiated. For irradiation,  
146 ephippia were placed on the bottom of a conical plastic Eppendorf microtube (1.5 mL)  
147 containing 0.5 mL of water. To ensure different radiation doses, we changed either the duration  
148 of exposure (sources 1 and 3) or the distance from the radiation source (source 2). The dose rate  
149 calculations were based on the activity of the  $^{137}\text{Cs}$  sources. For the source 1 they were verified  
150 by direct measurements with a DKG-02U dosimeter (SPC “Doza” Ltd, Russia). For the source  
151 2 dosimetry was undertaken at distances of 46 and 100 cm from the source, it has confirmed the  
152 dose rate  $\dot{D}_0 \approx 38$  mGy/h when recalculated to  $R_0 = 15$  cm. Then the distances  $R_i$  to obtain the  
153 doses  $D_i$  were calculated for  $t = 48$  hours of irradiation as:

$$154 \quad R_i = R_0 \sqrt{\dot{D}_0 \frac{t}{D_i}}, \quad (1)$$

155 The inaccuracy in the estimated high radiation doses (80–100 Gy) for Source 2 can be  
156 bigger than in case of small doses, due to closer location of samples to the source of radiation. .

157 An ILU-6 industrial high-frequency electron accelerator (Institute of Nuclear Physics SB  
158 RAS, Novosibirsk) was used to obtain high doses of radiation within a relatively short period of  
159 time. For irradiation at accelerator, we adjusted electron beam parameters to 2.4 MeV energy,  
160 320 mA pulse current and repetition rate 25 Hz. Tantalum bremsstrahlung converter 0.6 mm

161 thick was used. The dose rate in place of sample location measured beforehand by  
162 thermoluminescent dosimeters was equal to 0.5 Gy/s. To protect samples and  
163 thermoluminescent dosimeters from the scattered electrons, we used a metal container with 6  
164 mm thick walls. The spectrum of photons from the conversion target can be characterized by  
165 energies of up to 2.4 MeV. The average part of the spectrum is equal to ~ 0.5 MeV (while the  
166 Cesium line energy of gamma radiation is 0.66 MeV). Thus, the bremsstrahlung photons  
167 produced by the accelerator ILU-6 with the initial beam of electrons with an energy of 2.4 MeV  
168 can be considered as relatively close to the  $^{137}\text{Cs}$  source of radiation.

169 Resting eggs were irradiated in the dark at 4– 10°C. The absorbed doses from the point  
170  $^{137}\text{Cs}$  sources or accelerator, the time during which the absorbed doses were accumulated, and  
171 the characteristics of resting eggs used for the experiments are summarized in Table 1.

172

### 173 *2.3. Hatching success of irradiated resting eggs*

174 After irradiation, eggs were placed into the climate chamber for hatching. For each dose  
175 and source of radiation, we placed eggs into 500 mL jars with *Chlorella* as food at a  
176 concentration of 400 thousand cells/mL. Such conditions were determined as favorable for eggs  
177 reactivation with almost 100% hatching success for untreated resting eggs. Every three days,  
178 the medium was renewed. From the first day until the end of egg reactivation, neonates hatched  
179 from the eggs was counted and removed from jars daily. Removed animals were used for life-  
180 cycle experiments (see section 2.4). The peak of hatching usually was observed on days 3-5 of  
181 reactivation. However, we monitored the hatching success for two weeks. The hatching success  
182 was calculated as the ratio of hatched eggs to the total number of eggs in the experiment.

183

### 184 *2.4. Life-table experiments with females hatched from irradiated eggs*

185 For each irradiated portion of eggs, 15-20 randomly selected neonates (size 0.45–0.65 mm, <24  
186 h old) were placed individually in 20 mL of medium with food concentration of 200 thousand  
187 cells/mL to perform life-table experiments. The medium in life table experiments was renewed  
188 daily. Life-table experiments continued until the death of all experimental animals. The food  
189 concentration of 200 thousand cells/mL was used to provide food conditions that did not limit  
190 the growth and parthenogenetic reproduction of females (Zadereev and Gubanov, 1996). This  
191 experimental protocol both does not contradict the optimal conditions for toxicity test with  
192 *M. macrocopa* (Oh and Choi, 2012) and our numerous previous experiments that proved this  
193 food level and growth conditions to be optimal for life-cycle studies with *M. macrocopa*  
194 (Zadereev and Gubanov, 1996).

195 For each female, we measured, under 16x magnification, the body length (L, mm) on  
196 the first day of the experiment ( $L_0$ ) and the day before it produced the first clutch ( $L_{fin}$ ). The  
197 body lengths were used to calculate the specific growth rate of juvenile females (g):

$$198 \quad g = \frac{(\ln L_{fin} - \ln L_0)}{t}, \quad (1)$$

199 where t is interval between measurements (days).

200

201 For each female, we counted the number of offspring in each clutch, determined the sex  
202 of the progeny, and recorded the time of death of the female (in days). Average lifespan,  
203 average number of produced clutches and average proportion of males in the progeny were  
204 calculated for each dose. Data on the fecundity and mortality were used to calculate the net  
205 reproductive rate ( $R_0$ ) (Krebs, 1985):

$$206 \quad R_0 = \sum_{x=0}^{\infty} l_x \cdot m_x, \quad (2)$$

207 where  $l_x$  is the proportion of animals that survived until age  $x$ ,  $m_x$  is average fecundity at  
208 age  $x$ .

209

### 210 *2.5. Population experiments.*

211 Resting eggs that were irradiated by source 2 were used to initiate population  
212 experiments. We used resting eggs that absorbed doses of 2, 10, 50, 80 and 100 Gy. Eggs were  
213 hatched under the conditions described above (see section 2.3). For each dose, 15 randomly  
214 selected neonates (size 0.45–0.65 mm, <24 h old) were used to initiate 3 experimental  
215 populations (5 females per population). Females hatched from the control group of eggs were  
216 used to initiate three control populations. Populations developed in the batch culture in 500 mL  
217 experimental vessels with the continuous renewal of the medium (the flow-through rate – 500  
218 mL/day). *Chlorella* concentration in the medium was adjusted to 200 thousand cells/mL.  
219 During population experiments, for each population, every three days, we determined the total  
220 number of animals, size and sex of all animals and the mode of reproduction – parthenogenetic  
221 or gametogenetic – for adult females. We also calculated the number of resting eggs produced  
222 by populations. These resting eggs were removed from experimental vessels. Population  
223 experiments were run for 15 days (5 measurements). Thus, for each observation date we had  
224 the following characteristics for each population: total number of animals, numbers of juvenile,  
225 parthenogenetic and gametogenetic females and males, number of produced resting eggs.

226

## 2.6. Statistical analysis

The effect of irradiation dose and source of radiation on the hatching success of resting eggs was estimated by multiple regression analysis with the dose and source of radiation as independent variables and proportion of hatched eggs as depended variable.

To test the effect of the dose on the lifespan, somatic growth rate of juvenile females, number of produced clutches and proportion of males in the progeny for each source of radiation we used nonparametric comparison of multiple independent samples (Kruskall-Wallis test) that was followed by multiple comparisons to determine the difference between different doses and control.

To estimate the sensitivity of the life cycle parameters we calculated mean, median, coefficient of variation, minimal and maximal value and standard error for the controls, values of parameters for the range of doses up to 2 Gy and values of parameters for the range of doses from 10 to 100 Gy).

The effect of the dose on the net reproductive rate for each source of radiation was estimated by multiple regression analysis with the dose as independent variable and net reproductive rate as depended variable. To estimate the effective dose that induces 50% reduction in net reproductive rate ( $ED_{50}$ ) we pulled data from all sources, and calculate  $ED_{50}$  based on linear approximation of pulled data. The accuracy of linear approximation ( $R^2$ ) was equal to 0.83.

The effect of dose on the size of populations and numbers of juvenile females, males, parthenogenetic and gametogenetic females were estimated for each date of observations separately with one-way ANOVA. The effect of the dose on the total number of resting eggs produced by populations was estimated with one-way ANOVA. All statistical calculations were performed in STATISTICA 8.0.

## 3. Results

### 3.1. Hatching success of irradiated resting eggs

In the range of doses from the background level to 100 Gy, the effect of gamma radiation on the hatching success of resting eggs of *M. macrocopa* was non-significant (Multiple regression,  $p=0.10$ ). The effect of the source of gamma radiation on hatching success was also non-significant (Multiple regression,  $p=0.79$ ). The average hatching success in the range of doses from the background level to 100 Gy was  $89\pm 6\%$  of irradiated eggs. The dose of gamma radiation of 200 Gy resulted in the 100% mortality of irradiated resting eggs of *M. macrocopa* (Fig. 1).



### 3.2. Life cycle parameters of animals hatched from irradiated eggs

Table 2 summarizes the characteristics of life cycle parameters of animals from control groups, animals hatched from eggs irradiated with doses up to 2 Gy and animals hatched from eggs irradiated with doses in the range of 10-100 Gy.

*The life span of females* hatched from irradiated eggs did not differ from the life span of control animals for all sources of radiation (Fig. 2A).

*The somatic growth rate of juvenile females (GRJ)* hatched from irradiated eggs did not differ from the GRJ of control animals for sources 1 and 2. However, for source 3, the GRJ of animals hatched from eggs with the absorbed dose of radiation 40 and 100 Gy was significantly lower than GRJ of control animals (Kruskal-Wallis test:  $p < 0.0001$ ) (Fig. 2B).

*The number of hatched clutches* was the most sensitive parameter of the life cycle of females hatched from irradiated eggs. For the source 1 the effect of dose on the number of hatched clutches was not significant. The reproduction of females was strongly suppressed by the high doses of irradiation for the sources 2 and 3. The number of clutches produced by females hatched from eggs with the absorbed dose of radiation of 80 and 100 Gy was smaller than the number of clutches from females hatched from the eggs with smaller absorbed doses or control animals (Kruskal-Wallis test:  $p < 0.0001$ ) (Fig. 2C).

*The proportion of males* in the progeny of females hatched from irradiated eggs did not differ from the proportion of males in the progeny of control animals for all sources of radiation (Fig. 2D).

*Net reproductive rate.* The effect of the dose on net reproductive rate was not significant for the source 1 (Multiple regression,  $p = 0.34$ ) and significant for sources 2 and 3 (Multiple regression,  $p < 0.001$  and  $p = 0.037$  respectively). The estimated  $ED_{50}$  (effective dose that induces 50% reduction in net reproductive rate) was 50 Gy (Fig. 3).

### 3.3. Population experiments

The effect of the irradiation of resting eggs was also observed on the population level. We observed the effect of dose both for the size of the population and for the structure of the population. The size of the population that had been initiated from females hatched from resting eggs exposed to 80 Gy was significantly smaller than size of the control population for days 4 and 7 but after the population recovered and reached the maximal size equal to other populations. The size of the population that had been initiated from females hatched from resting eggs exposed to 100 Gy was significantly smaller than size of the control population for all days of observations (Fig 4A).

296 The effect of doze of irradiation on the structure of the population was detected for  
297 numbers of juvenile and parthenogenetic females (Table 3). At the first days of observations  
298 the number of juvenile females in control population was higher than in populations that had  
299 been initiated from females hatched from resting eggs exposed to 80 (days 4 and 7) and 100 Gy  
300 (days 4, 7 and 10). The number of parthenogenetic females in the population that had been  
301 initiated from females hatched from resting eggs exposed to 80 Gy was smaller than in the  
302 control only for the day 7 of observation, in the population that had been initiated from females  
303 hatched from resting eggs exposed to 100 Gy – for all days of observations except day 4 (Fig. 4  
304 B and C). The numbers of males and gametogenetic females did not differ between different  
305 treatments and control (Fig. 4 D and E).

306 The number of resting eggs produced in the population is a key parameter that  
307 characterizes its development. The effect of the dose was marginally significant for this  
308 parameter (ANOVA,  $F(5, 12)=3.1828$ ,  $p=0.046$ ). However post-hoc Fisher test demonstrated  
309 that number of produced resting eggs in none of treatments significantly differ from number of  
310 produced resting eggs in control populations. (Fig. 5).

311

## 312 **4. Discussion**

### 313 *4.1. The effect of radiation on resting eggs*

314 We determined the dose-response relationship for the effect of gamma radiation on  
315 hatching success of resting eggs of *M. macrocopa*. The absorbed dose of 200 Gy was lethal to  
316 resting eggs. Gamma irradiation in the range of doses from the background level to 100 Gy had  
317 no effect on survival of resting eggs.

318 Studies of the effect of ionizing radiation on resting eggs of cladocerans are scant. An  
319 experiment was performed at the International Space Station (ISS) to test the ability of resting  
320 eggs of *Daphnia* sp. to endure the outer space. Despite the high temperature differences and the  
321 impact of ionizing radiation (absorbed dose was equal to 2–3 Gy), part of the resting eggs  
322 retained the ability for reactivation (Novikova et al., 2011). Generally speaking, we should  
323 expect relatively high tolerance of resting eggs to the effect of radiation. It was noted that  
324 radiosensitivity correlated positively with the rates of metabolic processes, which resulted in  
325 the tolerance to the effect of radiation of dormant eggs of aquatic invertebrates (Eisler, 1994) or  
326 animals in cryptobiosys (Watanabe, 2006). Some anhydrobiotic invertebrates show extremely  
327 high tolerances against radiation. A tardigrade, *Macrobotus areolatus*, tolerates exposure to  
328 5500 Gy of X-ray (May et al., 1964). It was determined that LD<sub>50</sub> for dry *Artemia* cysts can be  
329 as high as 5000 Gy of Co<sup>60</sup> gamma radiation (Iwasaki, 1964).

330 Even some active invertebrates can tolerate high doses of radiation. It was found that  
331 rotifers of class *Bdelloidea* could tolerate doses of up to 600 Gy (Gladyshev and Meselson,  
332 2008). This tolerance was also related to the ability of rotifers to survive desiccation.  
333 Gladyshev and Meselson (2008) suggested that the extraordinary radiation resistance of  
334 bdelloid rotifers was a consequence of their ability to enter the resting stage to survive episodes  
335 of desiccation encountered in their habitats. The damage incurred in such episodes includes  
336 DNA breakage that is repaired upon rehydration. Thus, these species have developed effective  
337 mechanisms of DNA repair that can be activated to minimize the negative effects of radiation.

338

#### 339 4.2. The effect of irradiation of resting eggs on the individual responses of active animals

340 Even though survival and hatching of resting eggs in our experiments was not affected  
341 by doses below 100 Gy, the effect of radiation was manifested in hatched neonates at the  
342 individual and population levels. Negative effects on life cycle parameters were observed only  
343 in the range of high doses. In the range of low doses (up to 2 Gy), none of the life cycle  
344 parameters (lifespan, somatic growth rate, number of clutches, proportion of males in progeny,  
345 net reproductive rate) of hatchlings was affected by the irradiation of the resting stage. In the  
346 range of high doses (up to 100 Gy), the effect of the dose on life cycle parameters was  
347 significant. The most sensitive parameter was the net reproductive rate.

348 We can estimate the sensitivity of different life cycle parameters based on the  
349 comparison of coefficients of variance for values of life cycle parameters in the control and in  
350 the low dose and high dose ranges of radiation. Lifespan and growth rate of juvenile females  
351 had similar and relatively small CV for all ranges of doses and in the control. Thus, they were  
352 relatively non-sensitive parameters. The proportion of males in the progeny had high variance  
353 for all ranges of doses and in the control. Thus, this is a highly variable parameter, which will  
354 be difficult to use as a sensitive endpoint. The coefficient of variance for the number of  
355 clutches was also high for all ranges of doses and in the control. The net reproductive rate had  
356 low variance in the control and in the range of low doses but high variance in the range of high  
357 doses. Thus, this parameter can be used as a sensitive endpoint in the life cycle of *Moina*.

358 Our results showed that, in cladocerans, reproduction parameters of active animals were  
359 the most sensitive parameters of life cycle in response to the effect of ionizing radiation on  
360 animals during the resting stage. We obtained similar results in several experiments with active  
361 animals exposed to ionizing radiation. For example, the size of clutches hatched by *Daphnia*  
362 *magna* decreased at radiation doses greater than 0.1 Gy. At the same time, radiation doses of 1–  
363 2 Gy did not affect either survival or somatic growth rate of females (Gilbin et al., 2008). A  
364 recent study by Sarapultseva and Dubrova (2016) also demonstrated that the fertility of

365 *Daphnia* significantly decreased at a dose of 0.1 mGy and at higher doses while the survival  
366 was significantly compromised only for *Daphnia* exposed to 1 and 10 mGy of acute  $\gamma$ -rays.  
367 Moreover, Alonzo et al. (2016) modelled population responses to chronic external gamma  
368 radiation in 12 laboratory species (including aquatic and soil invertebrates, fish and terrestrial  
369 mammals) and found that net reproductive rate showed the lowest EDR<sub>10</sub> (effective dose rate  
370 inducing 10% effect) in all species.

371

#### 372 4.3. The effect of irradiation of resting eggs on the population responses of active animals

373 A recent study demonstrated the transgenerational effect of parental exposure to  
374 ionizing radiation on survival and fertility of directly exposed *Daphnia magna* females and  
375 their offspring (Sarapultseva and Dubrova, 2016). The irradiations affected viability, fertility  
376 and the number of broods of irradiated *Daphnia* and their first-generation progeny. Results  
377 obtained by Sarapultseva and Dubrova (2016) also demonstrated substantial recovery of the F2  
378 progeny of irradiated F0 *Daphnia*.

379 We did not test the transgenerational effect in life table experiments with individuals.  
380 However, we performed population experiments that lasted several times longer than the  
381 generation time for *M. macrocopa*. It takes about three days for the female of *M. macrocopa* to  
382 mature and produce the first parthenogenetic clutch. As we continued population experiments  
383 for 15 days, we could expect that populations comprised at least four generations of females  
384 that were started from irradiated resting eggs. Thus, for the population experiments, we can  
385 attribute the observed differences to both maternal and transgenerational effects of radiation on  
386 resting eggs.

387 Population performance was affected by the irradiation of the resting stage of animals  
388 that initiated population. The size of the population that was initiated by hatchlings from resting  
389 eggs exposed to 100 Gy was smaller and the proportion of males in the population higher. The  
390 most pronounced population effect was the reduced number of resting eggs produced by the  
391 populations that had been initiated by hatchlings from resting eggs exposed to 100 Gy.

392 Based on the conceptual model of biological responses to different dose rates of  
393 ionizing radiation (Polikarpov, 1998), the dose rates that produce damage to populations and  
394 communities should be higher than the dose rates that produce detectable effects in individual  
395 organisms, which will be masked and eliminated at the level of the population or community.  
396 Responses to the acute exposure of the resting stage detected in our experiments at the  
397 individual and population levels were consistent with the conceptual model by Polikarpov  
398 (1998). The estimated ED<sub>50</sub> for the R<sub>0</sub> at the individual level was 50 Gy. However, populations  
399 initiated from resting eggs exposed to 50 Gy did not differ in size from the control population;

400 populations initiated from resting eggs exposed to 80 Gy demonstrated reduced numbers in the  
401 initial stage of development but, later, the population size recovered. Population responses were  
402 most probably masked by the individual variability, and recovery of the subsequent progeny of  
403 F0 females irradiated in the resting stage may have contributed to the success of the population.

404

#### 405 *4.4. The effects of different radiation sources*

406 We used radiation sources of different power and different nature (two  $^{137}\text{Cs}$  sources and  
407 an industrial electron accelerator). As a result, the same radiation doses were accumulated at  
408 different durations of exposure to ionizing radiation. We selected the duration of exposure or  
409 distance to the source of radiation so as different sources yielded the same radiation doses. The  
410 effect of the source of radiation on the life cycle parameters of animals was manifested in the  
411 range of high doses (from 10 to 100 Gy). For the same absorbed dose, the effect of the electron  
412 accelerator (higher dose rate) was more pronounced than the effect of the  $^{137}\text{Cs}$  source (lower  
413 dose rate). For example, for the absorbed dose of 100 Gy, none of the hatchlings from eggs  
414 irradiated with the electron accelerator reproduced while some of hatchlings from eggs  
415 irradiated with the  $^{137}\text{Cs}$  source produced several clutches.

416 Copplestone et al. (2001) noted that to develop environmental protection strategies, it is  
417 important to consider the relative biological effectiveness of different radiation sources. Our  
418 research was not aimed at comparing different dose rates and durations of exposure that  
419 resulted in the same absorbed dose. However, based on our results, we can conclude that the  
420 higher dose rate tends to produce more pronounced effects on life cycle parameters, which  
421 confirms similar observations on other aquatic invertebrates (e.g. Blaylock, 1971).

422

423 Data on the effect of ionizing radiation on aquatic invertebrates are fragmentary and  
424 incomplete (Dallas, 2012). Our work covers a substantial gap in this field of research. We  
425 determined the lethal dose of gamma radiation for resting eggs of cladoceran *M. macrocopa* and  
426 studied the delayed effects manifested in active animals at the individual and population levels.  
427 Resting eggs remain viable in bottom sediments for years. Thus, even sediments contaminated  
428 with anthropogenic radionuclides of low activity may produce a cumulative dose that will  
429 affect either hatching of resting eggs or life cycle parameters of hatchlings.

430 In the present study, we investigated the effect of gamma radiation on resting eggs.  
431 There are studies focused on the effects of acute and chronic radiation exposure on the life  
432 cycle of active animals. There is one knowledge gap that needs to be filled. We demonstrated  
433 that during diapauses, resting eggs can tolerate high doses of radiation. Presumably, resting  
434 eggs are very sensitive to the effect of radiation at the early embryonic stage (Donaldson and

435 Foster 1957), e.g. during hatching. It takes several days for the embryo in the resting egg to  
436 develop and hatch. During this period, the embryo should be very sensitive to the effect of  
437 radiation. In the case of contamination of bottom sediments, acute radiation exposure at the  
438 stage of reactivation of resting eggs will probably be important for the development of  
439 zooplankton. This effect should be investigated in a separate study, in order to understand the  
440 complex effects of radiation on the species with cyclic reproduction.

441

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529 *Moina macrocopa*. *Dokl. Biochem. Biophys.* 466, 61–65.



530 Table 1. Conditions of experiments on irradiation of resting eggs of *Moina macrocopa*.

Dose power	Distance to source, cm	Irradiation time, h	Absorbed dose, Gy	Duration of resting state of eggs before experiment, months
Source 1		45	0.19	
<sup>137</sup> Cs source		95	0.39	
4.15 mGy/h	1.5	212	0.88	12
(at the distance		404	1.67	
of 1.5 cm)		504	2.09	
		570	2.37	
Source 2	20.2		1	
<sup>137</sup> Cs source	14.3		2	
38 mGy/h	6.4		10	15
(at the distance	4.5	48	20	
of 15 cm)	3.2		40	
	2.3		80	
	2.0		100	
Source 3		0.0056 (20 sec)	10	
Accelerator		0.011 (40 sec)	20	
0.5 Gy/s	Not relevant	0.022 (80 sec)	40	15
(at the distance		0.056 (200 sec)	100	
of the sample		0.111 (400 sec)	200	
location)				

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540 Table 2. Data on the selected life history variables of females of *Moina macrocopa* hatched  
 541 from irradiated resting eggs. Average lifespan (LS, days), growth rate of juvenile females  
 542 (GRJ, day<sup>-1</sup>), number of produced clutches (NC, clutches female<sup>-1</sup>), proportion of males in the  
 543 progeny (PM, %), net reproductive rate (R<sub>0</sub>, offspring female<sup>-1</sup>) Low - the range of doses from  
 544 the background level to the 2 Gy. High - the range of doses from 10 to 100 Gy. S.E. – standard  
 545 error, CV – coefficient of variance, Max – maximal value, Min – minimal value, N – number of  
 546 females tested.

Life history variable	The range of doses	Mean	S.E.	CV	Max	Min	N
LS	Control	9.41	0.44	35.37	17	4	58
	Low	10.61	0.29	35.99	20	4	175
	High	9.49	0.25	35.81	18	3	178
GRJ	Control	0.34	0.01	14.14	0.49	0.26	58
	Low	0.37	0.01	12.42	0.46	0.20	175
	High	0.32	0.01	20.08	0.51	0.10	176
NC	Control	3.31	0.25	58.59	8	1	58
	Low	3.71	0.17	60.11	9	0	175
	High	2.74	0.18	86.64	8	0	172
PM	Control	0.21	0.03	107.26	0.75	0	58
	Low	0.24	0.02	91.29	1	0	169
	High	0.30	0.02	78.40	1	0	124
R <sub>0</sub>	Control	32.38	4.66	24.94	41.47	26.9	3
	Low	32.56	1.21	10.52	36.65	28.55	8
	High	20.28	5.28	78.18	42.47	0	9

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557 Table 3. Significance of the post-hoc ANOVA analysis for the difference in size and structure  
 558 of the population between control and experimental treatments for different observations days.

Day of observation	Size of the population		Number of juvenile females		Number of parthenogenetic females	
	80 Gy	100 Gy	80 Gy	100 Gy	80 Gy	100 Gy
Day 4	p=0.04	p=0.03	p=0.03	p=0.02		
Day 7	p=0.04	p=0.005	p=0.02	p=0.01	p=0.02	p=0.01
Day 10		p=0.003		p=0.01		p=0.004
Day 13		p=0.01				p=0.005
Day 16		p=0.02				p=0.0006

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583 **Figure captions:**

584

585 Fig. 1. The effect of gamma radiation from different sources (see Table 1 for details) on the  
586 survival of resting eggs of *M.macrocopa*.

587

588 Fig. 2. The effect of gamma radiation from different sources (see Table 1 for details) on the life  
589 cycle parameters of females hatched from irradiated resting eggs of *M.macrocopa*. A – lifespan,  
590 B - somatic growth rate of juvenile females, C - number of hatched clutches, D – the proportion  
591 of males in the progeny.

592

593 Fig. 3. Dose dependent reproduction of females hatched from irradiated resting eggs of  
594 *M.macrocopa*.

595

596 Fig. 4. Characteristics of the development of the experimental populations of *M.macrocopa*  
597 initiated from irradiated resting eggs. A – population size; B – proportions of different age and  
598 sex groups at different days averaged for all treatments; C – proportions of different age and  
599 sex groups in different treatments averaged for days.

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601 Fig. 5. Dose-dependent production of resting eggs by populations of *M. macrocopa* initiated  
602 from irradiated resting eggs.

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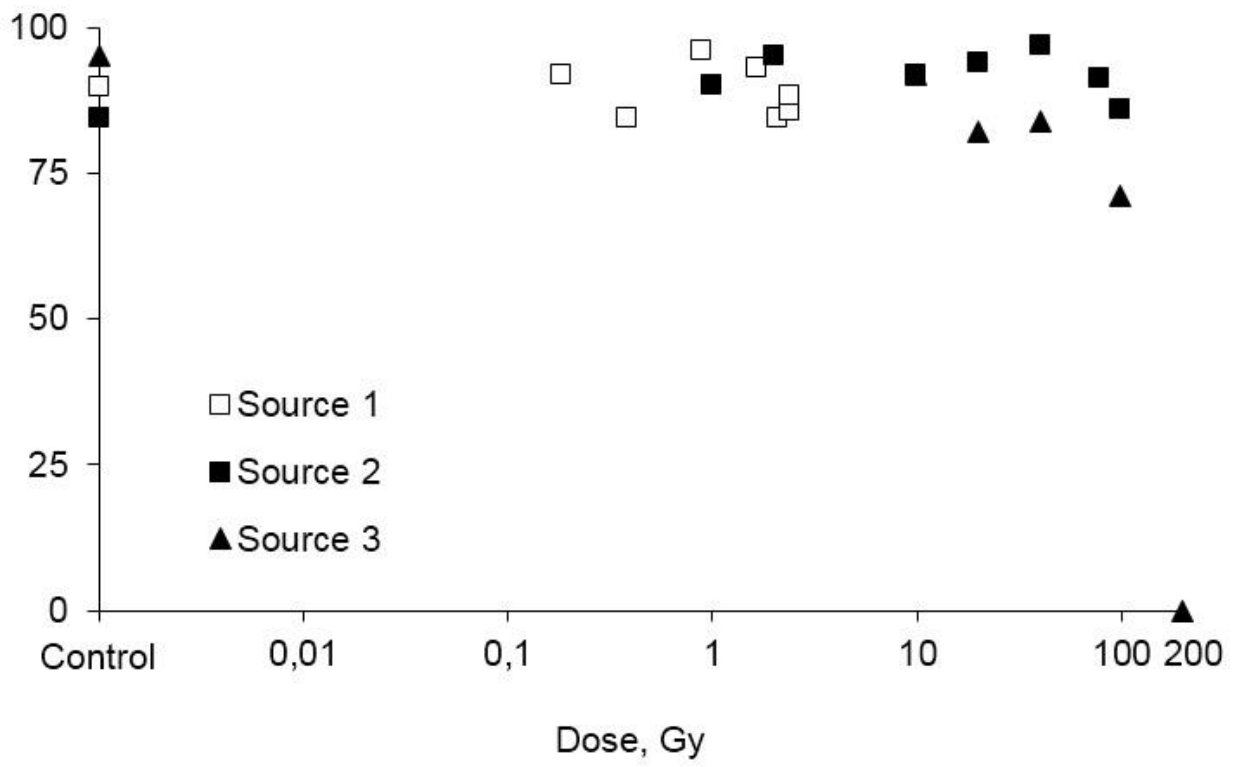
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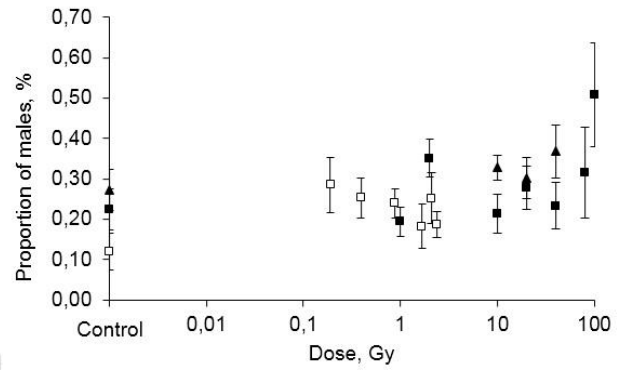
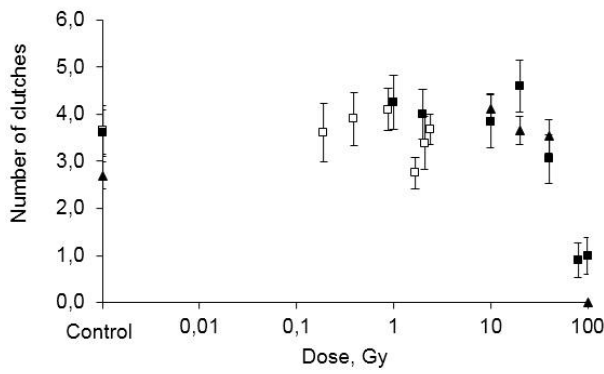
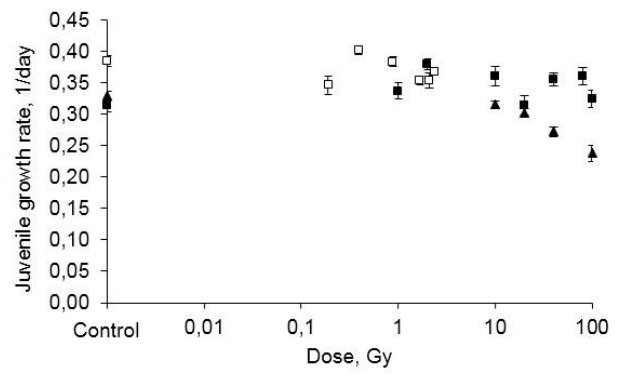
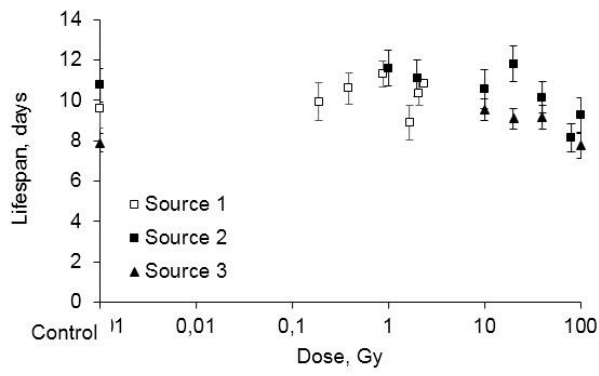
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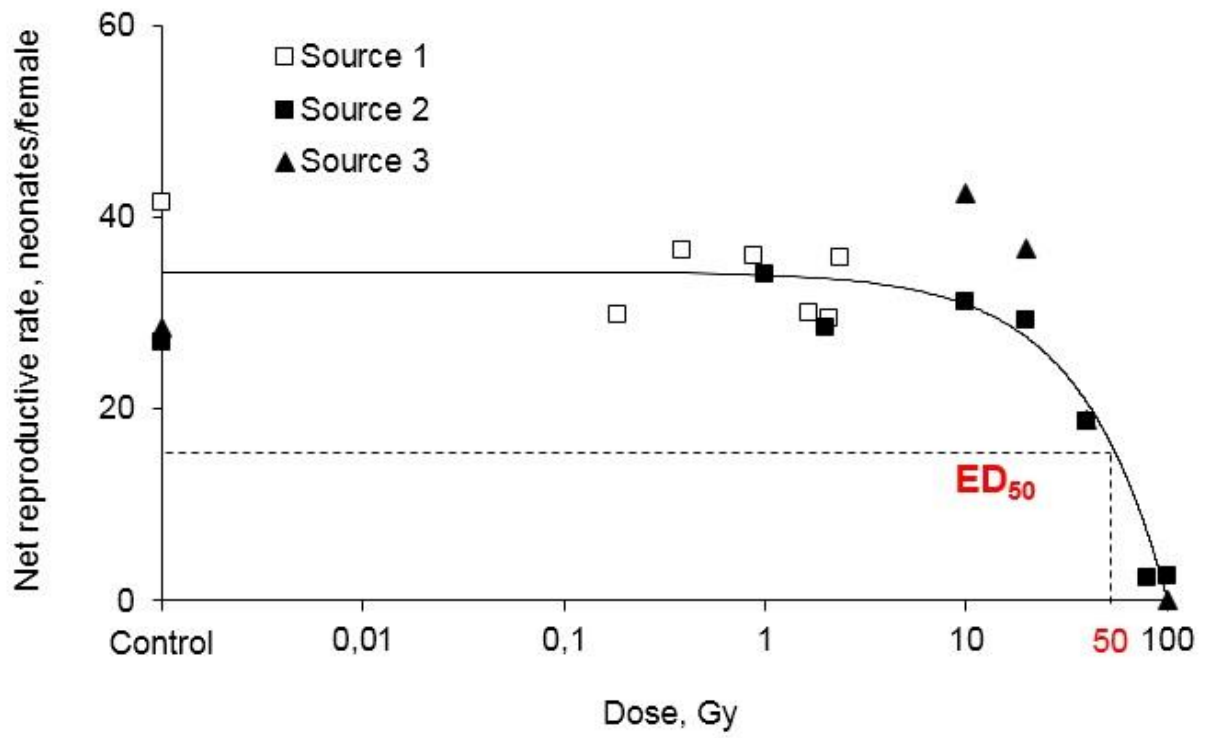
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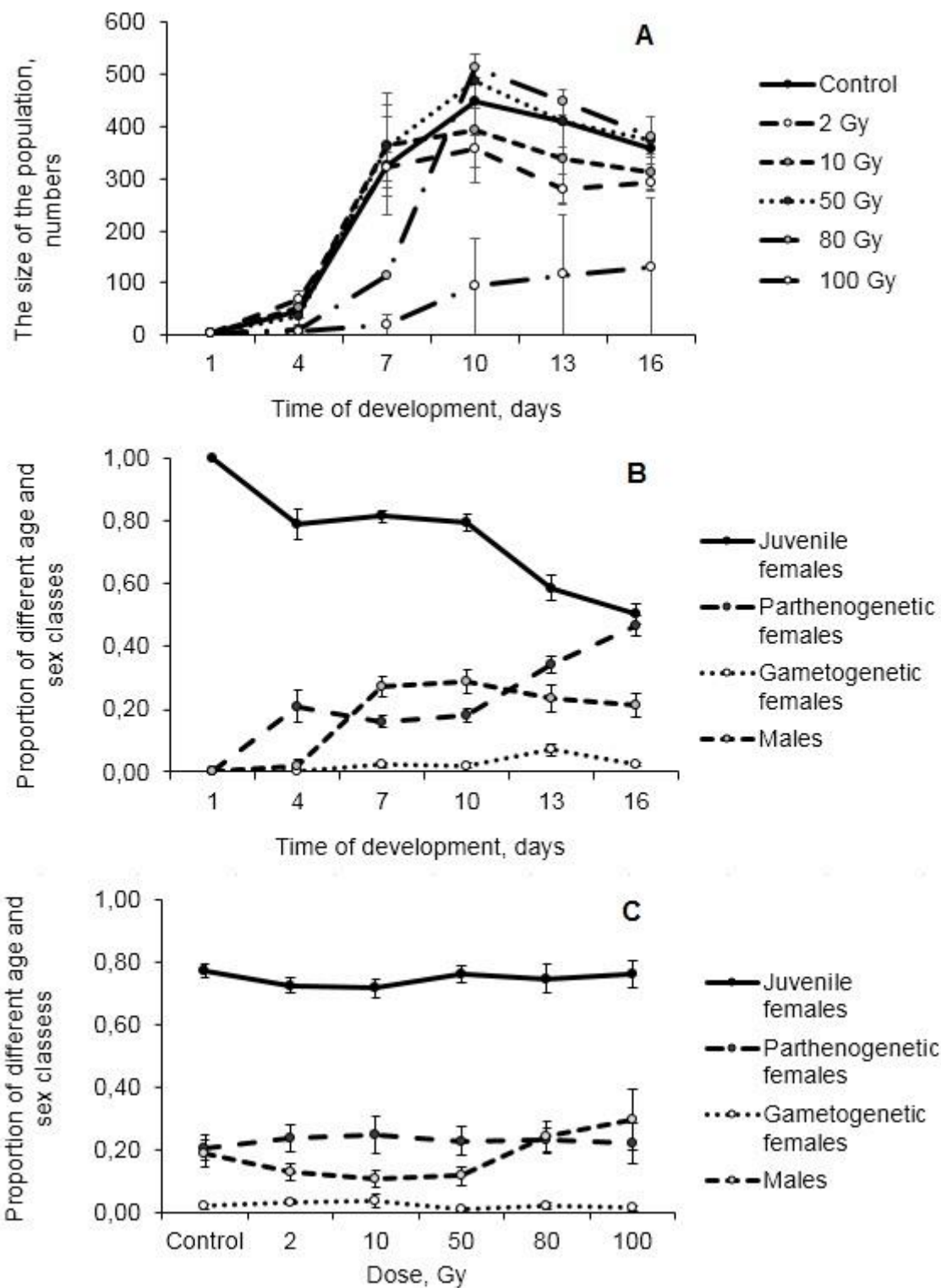
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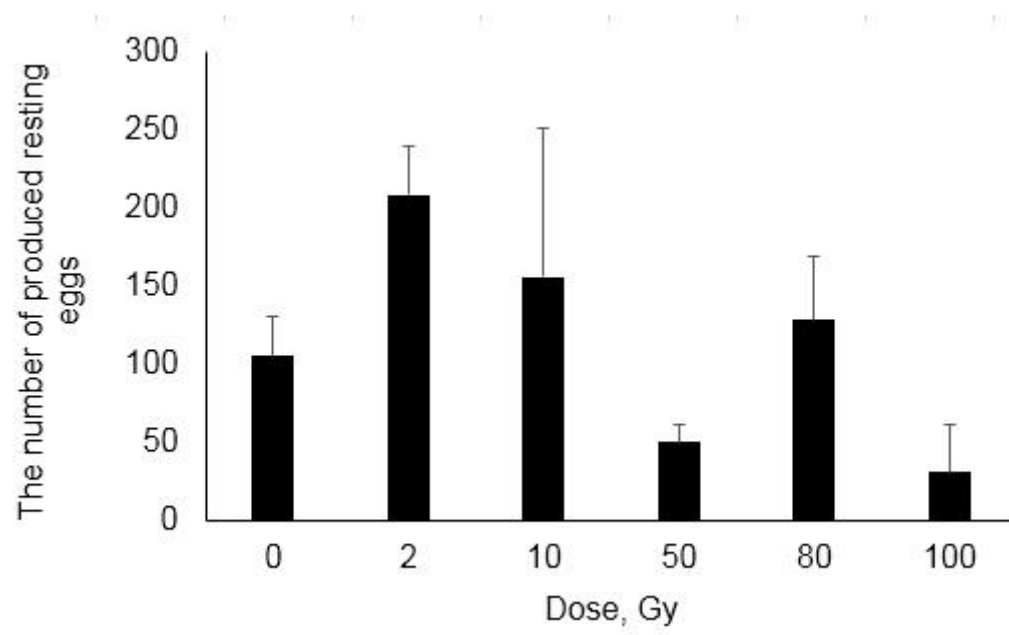
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