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41 **Running Head:** Irradiation leads to drastic effects in progeny

42 **Title:** Adverse effects induced by chronic gamma irradiation in progeny of adult fish not
43 affecting parental reproductive performance

44 **Abstract:** Multigenerational studies has become of great interest in ecotoxicology since the
45 consequence of parental exposure to contaminants on offspring generations was established *in*
46 *situ* or in laboratory conditions. This study mainly examined the chronic effects of external
47 Cs-137 gamma irradiation exposure at 4 dose rates (control, 0.5, 5 and 50 mGy h^{-1}) on adult
48 zebrafish (F0) exposed for 10 days and its progeny (F1) exposed or unexposed for 4/5 days.
49 The main endpoints investigated included parental reproductive performance, embryo-larval
50 survival, DNA alterations and ROS production in F0 and F1. No effects on reproductive
51 success, fecundity or egg fertilization rate were observed. However, drastic effects were
52 observed on F1 exposed to 50 mGy h^{-1}, resulting in a mortality rate of 100%. The drastic
53 effects were also observed when the progeny was not irradiated. It was demonstrated that the
54 sensitivity of the embryos was mainly due to parental irradiation. Moreover, these drastic
55 effects induced by adult irradiation disappeared over time when 10 d- irradiated adults were
56 placed in a non-irradiated condition. DNA alterations in larvae were observed for the three
57 dose rates, and an increase of ROS production was also shown for the two lowest dose rates.
58 This study improves our understanding of the consequences of parental exposure conditions
59 to the progeny. Furthermore, it provides an incentive to take transmitted generational effects
60 into account in ecological risk assessments.

61

62 **Keywords:** reproduction, irradiation exposure, zebrafish, risk assessment

63 **INTRODUCTION**

64 Gamma radiation represents a potential health risk to biota, due to its ability to ionize
65 molecules in tissue. Ionizing radiation is known to induce oxidative stress, DNA damage and
66 apoptosis, which therefore constitute usual molecular markers for evaluating toxicity
67 mechanisms(Gagnaire et al. 2015; Jaafar et al. 2013; Knowles 2002; Praveen Kumar et al.
68 2017; Simon et al. 2011b; Sinha et al. 2018). Significant effects in animals (fish, nematode,
69 Daphnia) in terms of survival, reproduction and development have been observed (Adam-
70 Guillermin et al. 2012; Buisset-Goussen et al. 2014; Gagnaire et al. 2015; Knowles 2002;
71 Parisot et al. 2015; Simon et al. 2011b). Moreover, damage effects in fish have mainly been
72 observed for early life stages, which are considered as the most vulnerable to ionizing
73 radiation. Embryogenesis, in addition to gametogenesis and organogenesis, can be affected by
74 ionizing radiation due to the high rate of cell division, proliferation and differentiation
75 (Hurem et al. 2017a; Hurem et al. 2018) and enable the consequences of irradiation to be
76 assessed.

77

78 In terms of ecological risk assessment (ERA), endpoints on the impact on adult reproduction
79 performance (reproductive success, fecundity, fertility) and on development of early stages
80 (growth, survival) remain the main useful endpoints in characterising the ecological
81 consequences of pollutants, including ionizing radiation. Radiological protection criteria are
82 largely based on data from acute exposure experiments of adult organisms, thus information
83 on the effects of ionizing radiation during sensitive life stages and after chronic exposure are
84 lacking (Hurem et al. 2017a; Hurem et al. 2017b). Overall, few data are available concerning
85 the effects of ionizing radiation in fish. The ecological screening benchmark for a generic

86 ecosystem of 10 μGy h^{-1} based on the HDR_5 (Hazardous Dose Rate for 5% of the species) for
87 radioactive substances was built with five EDR_{10} (Effect Dose Rate related to a change of
88 10% for a particular effect) from fish, which involved values ranging from 47 to 20,881 μGy
89 h^{-1} (Garnier-Laplace et al. 2010). This generic benchmark dose rate was proposed by the
90 European project, ERICA, for the screening of potential radiological effects (Brown et al.
91 2008). For freshwater and chronic γ external exposure, applying a safety factor of 50 to the
92 lowest EDR_{10} (reproductive endpoint: 516 μGy h^{-1}) led to a predicted no-effect value of 10
93 μGy h^{-1} (Garnier-Laplace et al. 2006). Derived consideration reference levels (DCRLs) *i.e.*,
94 "the dose rate band within which there is likely to be some chance of deleterious effects
95 occurring to individuals of such type of a given type of Reference Animal and Plant (RAP)"
96 indicate a possible reduction of reproductive success for dose rates ranging between 40 and
97 4,000 μGy h^{-1} for trout (ICRP 2012).

98

99 Firstly, the EDR_{10} did not include the assessment of adult reproduction performance.
100 Secondly, none of these fish species belong to the cyprinid family. Thirdly, the consequence
101 of parental exposure for offspring generations have not yet been taken into account in the
102 ERA. However, multigenerational studies have become of great interest (Buisset-Goussen et
103 al. 2014; Hurem et al. 2017a; Lemos et al. 2017; Parisot et al. 2015). For fish, the progeny of
104 adult zebrafish exposed to 53 mGy h^{-1} (^{60}Co gamma radiation) for 27 days showed a 100 %
105 mortality rate occurring at the gastrula stage (Hurem et al. 2017a). Thus, parental exposure
106 can lead to hereditary effects in offspring, probably due to epigenetic mechanisms (Herráez et
107 al. 2017; Hurem et al. 2017a; Lemos et al. 2017).

108

109 As fish are known to be the most radiosensitive organisms among the poikilothermic
110 aquatic animals (Garnier-Laplace et al. 2006), zebrafish, or *Danio rerio*, were chosen in the
111 present research as a cyprinid model for assessing gamma radiation effects on reproduction
112 performance and larval development. Zebrafish have been widely used for examining effects
113 of ionizing radiation (Choi et al. 2010; Choi et al. 2015; Gagnaire et al. 2015; Hurem et al.
114 2017a; Hurem et al. 2017b; McAleer et al. 2005; Ng et al. 2017; Simon et al. 2011b; Yum et
115 al. 2010) in addition to transgenerational effects (Hurem et al. 2017a; Kamstra et al. 2018).
116 Indeed, breeding success, high fecundity and rapid development are the main advantages of
117 this model in performing multigenerational studies (Lawrence 2007; Simon et al. 2014).

118 In this study, adult zebrafish were exposed to four dose rates (0, 0.5, 5 and 50 mGy h^{-1},
119 ^{137}Cs gamma radiation) over 10 days. The dose rates were higher than those in "hot spots"
120 measured in freshwater ecosystems around Chernobyl (< 0.2 mGy h^{-1}) (Bonzom et al. 2016;
121 Fuller et al. 2018; Lecomte-Pradines et al. 2014; Lerebours et al. 2018). Reproduction was
122 initiated and the progeny were then exposed or not to the same dose rates over 96-120 hours.
123 The objectives of this study were to evaluate (i) the adult reproductive performance, (ii) the
124 effects in the progeny of irradiated parents and (iii) the responses at the molecular and
125 organism level. Effects on adults were assessed by reproductive performance and genotoxicity
126 (comet assay). Effects on the progeny were assessed using the survival rate (%) and the
127 genotoxic and oxidative stress effects. The progeny were also placed in non-irradiated
128 conditions (F1 recovery) over 120 hours to evaluate the impact of adult irradiation on the
129 progeny (generational effects).

130 Complementary objectives concerned the increase of the exposure duration on the effects
131 and the time necessary to obtain reproductive resilience: for the two lowest dose rates tested
132 (lowDR: 0.5, 5 mGy h^{-1}), adult F0 exposure duration was increased to 24 days; for the highest

133 dose rate (highDR: 50 mGy h^{-1}), 10 day-irradiated adults (F0) were placed in non-irradiated
134 conditions over 63 days (F0 recovery). Adult performance, progeny survival and molecular
135 effects were also evaluated during these complementary experiments.

136 **MATERIALS AND METHODS**

137 *Fish husbandry*

138 The project (APAFIS#15821) was authorized by the IRSN ethics committee N°81 (EU
139 0520) in an application under the directive 2010/63/UE relating to animal care. The study was
140 conducted on wild-type zebrafish that were kept, reproduced and irradiated in a zebrafish
141 housing system (Zebtec Tecniplast stand Alone, Varese, Italy) with recirculating fresh water.
142 Adult fish were acclimatised over 2-3 weeks to tap water + 20% demineralized water renewed
143 daily (Aquadem, Veolia, France) (pH=7.4 ± 0.2, conductivity = 398 ± 2 µS cm^{-1}, T=28.4 ±
144 0.3 °C), with a 14 h light/10 h dark cycle photoperiod. The fish were fed *ab libitum* twice a
145 day with commercial flakes (Tetramin®).

146 *Adult and embryo exposure*

147 Figure 1 shows the exposure duration and endpoints for the experiments. Nominal dose
148 rates were 0.5 and 5 mGy h^{-1} for the low dose experiment (lowDR) and 50 mGy h^{-1} for the
149 high dose experiment (highDR). As both experiments (lowDR and highDR) were not carried
150 out at the same time, control fish conditions were implemented for each experiment. Gamma-
151 rays were emitted from a ^{137}Cs source (444 GBq, 662 keV, IRSN-MICADO-Lab platform).
152 Dose rates were simulated using MCNP5 software and measured using thermoluminescent
153 dosimeters (Chiyoada Technologies, Japan) and the values represented between 91.1 and

154 100.8% and between 80 and 120% of the nominal low DR and high DR values, respectively.
155 Two control conditions were kept in a separate room (60-80 nGy h^{-1}).

156 The population density of adult fish was 0.7 L g^{-1}. For the low dose rate experiment
157 (lowDR), 20 adult fish per condition (female: 0.66 ± 0.13 g, n=24, male: 0.50 ± 0.065 g,
158 n=11) were irradiated over 10 and 24 days. For the high dose rate experiment (highDR), 24
159 adult fish per condition (female: 0.38 ± 0.06 g, n=6, male: 0.40 ± 0.07 g, n=6) were irradiated
160 over 10 days. To keep the radiation exposure as long as possible, the daily control of abiotic
161 parameters (pH, conductivity, Temperature), the animal welfare and the feeding process were
162 carried out in less than one hour, five times per week. During this hour, irradiation was
163 stopped in order to access to the tanks. Food was supplied to the fish twice a day by automatic
164 suppliers. During the weekends, the control of these parameters was achieved through the use
165 of cameras, allowing animal welfare to be monitored without interrupting the irradiation. The
166 preparation of reproduction and egg collection required stopping the irradiation during four
167 hours. After 24 d of exposure, the adults from lowDR were sacrificed and the gonads were
168 collected. After 10 d of exposure, six adults from highDR were sacrificed and the gonads
169 were collected. Six other adults were introduced into non-irradiation conditions (F0
170 Recovery).

171 For each reproduction event, embryos were obtained from 10 spawning genitors (*i.e.*
172 replicates) for lowDR and 12 genitors (*i.e.* replicates) for highDR per condition. Each group,
173 consisting of one male and one female, was placed in specific spawning aquariums to avoid
174 egg predation in the zebrafish housing system. Adults were irradiated during reproduction.
175 After spawning, the egg viability (3-4 hpf, hours post fertilisation) of each spawn was
176 confirmed when the blastula stage was reached. The embryos (2.6 ml/egg) were then
177 reintroduced into small beakers (Ø=9 cm) in the zebrafish housing system to be irradiated (at

178 the same dose rate as the adult groups (F1 irradiated group) or without irradiation (F1
179 Recovery group) and larvae were fed starting from 5 dpf (days post fertilisation) (artificial
180 commercial food ST-1, Aqua Schwarz GmbH). The culture medium of the embryos was the
181 same as that of the adults and 10% was replenished daily.

182 *Ecologically-representative endpoints for adults*

183 For the lowDR experiment, adult F0 reproductive performance (n=10 couples per condition)
184 was assessed after 10 and 24 days of exposure for both dose rates tested. Endpoints concerned
185 the reproductive success (number of couples that spawned), the fecundity (number of eggs per
186 female) and the egg survival rate at 3 hpf (fertilization rate). For the highDR experiment, F0
187 reproductive performance was assessed after 10 days of irradiation (n=12 couples per
188 condition) and after 6, 36 and 63 days in non-irradiated conditions (F0 Recovery, n=6
189 couples) (Figure 1).

190 *Ecologically-representative endpoints and oxidative stress analysis for progeny*

191 For both experiments, the progeny survival rate (%) was assessed after 24 and 48 h in the
192 control, irradiated and non-irradiated (recovery) conditions. Complementary experiments
193 consisted of irradiating 3 hpf-eggs at 50 mGy h^{-1} over 96 h (30 eggs from three spawns per
194 condition) to measure the survival rate.

195 For the lowDR experiment, two pseudo-replicates of 80 eggs originating from three spawns
196 per condition including the control, irradiated and non-irradiated conditions, were followed to
197 measure the progeny survival rate over 1, 2, 10 and 22 d. For the highDR experiment, 30 eggs
198 from six spawns of the three conditions were tracked over 5 d (120h).

199 For the highDR experiment, photographs of 72 hpf-larvae were recorded. Morphology
200 (whole body size, surface of yolk reserve), malformations and cardiac edema were then
201 assessed using software (Danioscope image analysis system version 10.0, Noldus). Analyses
202 were made on 10 72 hpf-larvae per condition after 10 d F0 exposure (F0Irr. 10d) and after F0
203 recovery at 6 days (T10d+R6d) and at 36 days (T10d+R36d).

204 Reactive oxygen species (ROS) production was measured in F1-progeny from the
205 lowDR experiment using a protocol adapted from Hurem et al, (2017) and Gagnaire et al.,
206 2015. Given the high larval mortality rate, no measurements were made during the highDR
207 experiment. ROS production was determined using the fluorescent probe (2', 7' –
208 dichlorodihydrofluorescein diacetate (H_2DCFDA, Sigma-Aldrich, St. Louis, USA)) in 10
209 larvae per condition (larvae exposed from 3-4 hpf to 4 or 10 days originating from 10 d-adult
210 and 24 d-adult reproduction). Through the oxidation of H_2DCFDA in dimethylsulfoxide
211 (DMSO, Sigma-Aldrich, St. Louis, USA) water by ROS (mainly H_2O_2) the molecule was
212 converted into 2', 7' dichlorodihydrofluorescein (DCF), which is highly fluorescent. The
213 reported wavelengths for the measurement of DCF fluorescence are 500 nm for excitation and
214 525 nm for emission (TECAN Infinite M1000, Switzerland).

215 *Genotoxic effects in progeny and adult*

216 The genotoxic effects induced by gamma irradiation in the developmental stages (1 d, 4
217 d, 10 d for lowDR and 1 d for highDR experiments) and in gonads (10 d, highDR experiment)
218 of zebrafish were evaluated using the alkaline comet assay as described in Sing et al, (1998)
219 (1988) with modifications (Simon et al. 2011a; Simon et al. 2018). After centrifugation (110
220 g, 10 min, 8 °C, Eppendorf, 5427R, Germany), pellets were suspended in 1 mL of L15-
221 HEPES and used immediately. Cells were counted on a Malassez cell and their viability was

222 assessed using trypan blue. Two hundred nucleoids per slide were analysed at ×400
223 magnification under a fluorescence microscope (Nikon Eclipse E600) equipped with a 515–
224 560 nm excitation filter. Comet pictures were analysed using Comet IV software (Perceptive
225 Instruments). This assay was performed on three pools of ten 24-hpf eggs, on 10 individual
226 96-hpf larvae and on three pools of whole gonads per sex and per condition. The Tail Moment
227 was defined as the percentage of DNA in the tail multiplied by the length between the centre
228 of the head and tail.

229 *Statistical analysis*

230 All data are presented as means ± SD with significance taken at $p<0.05$. A comparison
231 between the control and irradiation or recovery exposures was performed. Before the
232 statistical analyses, the normality and homogeneity of the variance were tested using the
233 Shapiro-Wilks and Fisher tests, respectively. The T-test and one or two ways-ANOVAs were
234 used when data were normally distributed. The non-parametric test (Kruskal-Wallis) was used
235 when data were not normally distributed.

236 **RESULTS**

237 There were no instances of adult fish mortality during the experimental periods (control,
238 low DR and high DR experiments).

239 *Cumulative doses after exposure conditions*

240 For the LowDR experiment, cumulative doses in adults ranged between 120 and 2880
241 mGy after 10 and 24 days of exposure, respectively. For the highDR experiment, the
242 cumulative dose in adults was 12 Gy (SD Table 1). For the progeny, maximal cumulative

243 doses ranged from 264 to 2640 mGy for the lowDR experiment and 6 Gy for the highDR
244 experiment (SD Table 1).

245 *Adult performance (reproductive success, fecundity, total egg number and 3hpf-egg mortality)*

246 For the highDR experiment, all females (n=12 per condition) spawned after 10 days of
247 exposure. For the lowDR experiment (n=10 per condition), reproductive success was 100%
248 and 80% for both the irradiated conditions and control conditions, respectively.

249 Figure 2 shows the fecundity (number of eggs per female) after 10 days of exposure for
250 both experiments. High inter-individual variability was observed in all conditions. A
251 significant difference (Kruskal-Wallis, p=0.03) was observed between 0.5 mGy h^{-1} (240 eggs
252 ± 132) and control (364 eggs ± 123) conditions. In the control LowDR, 43% of all spawns
253 ranged between 101 and 300 eggs and 57% of spawns had higher than 300 eggs. At 0.5 mGy
254 h^{-1}, 20% of all spawns produced less than 100 eggs. At 5 mGy h^{-1}, no significant difference in
255 fecundity (413 eggs ± 148) was observed even though 89% of spawns had more than 300
256 eggs. At the high dose rate (50 mGy h^{-1}), a change in the size of spawns was also observed,
257 with an increase in the number of spawning events (75%, 9/12), providing between 101 and
258 300 eggs compared to the control (42%, 5/12).

259 A significant difference was observed between both control fish fecundity (lowDR: 364 eggs
260 ± 123 per female (n=7) versus the highDR: 203 ± 117 eggs per female (n=12), p=0.042
261 Kruskal-Wallis test) and egg quantity distribution.

262 The quality of oocytes was assessed by the survival 3hpf-progeny, indicating the number of
263 fertilized *versus* non-fertilized eggs. The survival rate at 3 hpf remained high (>89%) without
264 any significant difference between exposure conditions (SD Figure 1). Over the 50 spawns

265 monitored, only two presented a survival rate of less than 80%. Almost 85% of the spawns
266 presented a survival rate of 90-100%.

267

268 Reproductive success after 24 days of lowDR exposure was not impacted by irradiation; 80,
269 90 and 100% of couples reproduced for the controls, 0.5 and 5 mGy h^{-1}, respectively. The
270 significant difference in fecundity previously observed after 10 days between the control and
271 0.5 mGy h^{-1} was not observed after 24 d of exposure. Fecundity was 375 ± 102, 289 ± 138
272 and 357 ± 138 eggs per female for the controls, 0.5 and 5 mGy h^{-1}, respectively. In the
273 controls, 37.5% of spawns contained between 101 and 300 eggs and 62.5% contained more
274 than 301 eggs, as observed after 10 days. At 5 mGy h^{-1}, 75 % of spawns presented more than
275 301 eggs, as previously observed after 10 days of exposure. The survival rate at 3 hpf-egg was
276 close to the one measured after 10 days of adult exposure.

277 *Progeny survival and size*

278 Figure 3 shows F1 progeny survival rate (%) measured at 24 and 48 h for all exposure
279 conditions of both experiments. For the lowDR experiment, the survival rate was higher than
280 87%, with no significant difference between the conditions. For the highDR experiment, a
281 significant difference was observed between the control and larvae exposed to 50 mGy h^{-1} for
282 24 h (p=0.02) and 48 h (p=0.006). Significant differences were also observed between the
283 control and F1 recovery conditions for both times (p=0.015 and p=0.006 for 24 and 48 h,
284 respectively). Figure 4 shows the survival rate (%) in F1 progeny originating from the lowDR
285 experiment. Survival rates were higher than 80% and 60% at 2 and 10 d, respectively, for all
286 exposure conditions. No significant effect of treatments and control was observed in the
287 progeny coming from 10 d-irradiated adults. Similar results were observed for the progeny

288 coming from 24 d-irradiated adults. A significant difference was observed between 5 and
289 F1R5 (Kruskal-Wallis test: p=0.049, n=6) at 22 days of exposure. No significant differences
290 were observed for the hatching rate (%) at 48 h (data not shown).

291 For the irradiated and non-irradiated (F1 Recovery) larvae (Figure 5), 100% died after
292 120 h of highDR exposure, although the average survival rate for the control condition was 84
293 ± 11%. In the control condition, the mortality rate was low and remained constant from 24
294 hpf. F1 irradiated (51%) and F1 Recovery (58%) survival rate at 24 hpf was low compared to
295 the control and ranged between 16 to 96%, indicating a high spawn variability (SD Figure 2).
296 Drastic effects seemed to appear more rapidly for F1 irradiated larvae than for F1 Recovery
297 larvae (SD Figure 2).

298 Figure 6 shows the survival rate (%) in F1 progeny coming from the highDR
299 experiment where adults were placed in non-irradiated exposure conditions (F0 Recovery)
300 over 63 days. Fish were induced to spawn after 6, 36 and 63 days. Drastic effects of adult
301 irradiation on survival progeny rate lasted for at least 6 days (35 ± 22, n=11 spawns) and until
302 36 days (48 ± 37, n=8 spawns). All larvae alive at 48 h from F0R0d, F0 R6d and F0 R36d,
303 died at 96 h. After 63 days in non-irradiated conditions, the progeny survival rate remained
304 high (>85%, n=11 spawns) and no significant difference was observed compared to the
305 control until 96 h.

306 For the highDR experiment, the body sizes of F1 irradiated and F1 Recovery larvae decreased
307 significantly compared to the control (Figure 7). Similar results were also observed for non-
308 irradiated 72 h-larvae originating from F0 recovery conditions after 6 days. After 36 d in non-
309 irradiated conditions (F0 R36d), the body sizes of non-irradiated progeny showed no
310 significant difference compared to the controls although 100% of mortality at 120 h was

311 observed. The surface of vitellus and pericardia area also showed no statistical difference with
312 the controls (data not shown).

313 *Oxidative stress*

314 Figure 8 shows ROS production in F1-progeny coming from the lowDR experiment.
315 Irradiation at 5 mGy h^{-1} led to an increase of ROS production in 4 d-exposed larvae coming
316 from 24 d-adult exposure. However, F1 R progeny exposed over 4 d at both dose rates
317 showed a significant increase in ROS production for larvae coming from 10 d- and 24 d-adult
318 exposure. No effects were observed in larvae (irradiated and recovery) exposed over 10 d
319 (Figure 10B).

320 *Genotoxic effects*

321 Figure 9 shows DNA damage measured in progeny coming from 24 d-adults in the
322 lowDR experiment, expressed by the tail moment. The progeny were placed in irradiated and
323 non-irradiated conditions (F1 Recovery) over 10 days. Significant DNA damage was noticed
324 at 4 d for irradiated (0.5 mGy h^{-1}: 11.3 ± 4.3%; 5 mGy h^{-1}: 14.4 ± 2.1%) and non-irradiated
325 larvae (F1R: 0.5 mGy h^{-1}: 8.4 ± 3%) compared to the controls (x10). At 10 d, the tail moment
326 showed no difference between the conditions.

327 Figure 10A shows DNA damage expressed by the tail moment in the progeny coming
328 from 10 d-adult fish from the highDR experiment. The progeny was collected in irradiated
329 and non-irradiated conditions (F1 Recovery) for 24 h. The high mortality rate did not allow
330 larval collection at 4 and 10 d. A significant increase of the tail moment was observed
331 between the control, irradiated (x6.5) and non-irradiated (x5) conditions. 10B-C shows DNA
332 damage in the gonads of males and females after 10 days of irradiation. DNA damage

333 increased for both sexes compared to the control. The effects were more pronounced for
334 males (x3) than for females (x1.7).

335 **DISCUSSION**

336 In the present work, embryo-larval and adult fish stages were exposed to 0.5, 5 and 50
337 mGy h^{-1} (^{137}Cs gamma radiation) to augment the dataset of gamma ray effects after parental
338 and progeny exposure.

339 *Zebrafish as a relevant model for multigenerational studies*

340 Reproduction was achieved for almost all couples and with a high egg survival rate (93
341 ± 7 % at 3 hpf for both control conditions, n=19), confirming the relevance of this model for
342 multigenerational studies (Lawrence et al. 2012; Simon et al. 2014). Differences in
343 reproductive performances were observed between the controls used in both lowDR and
344 highDR experiments. The body weights of the control adults in both experiments was
345 significantly different and could explain the differences in fecundity. Indeed, zebrafish
346 fecundity, and more generally reproduction performance, can be influenced by the quality of
347 the diet, the female body size and environmental enrichment (Karga et al. 2017; Lawrence
348 2007; Lawrence et al. 2012; Wafer et al. 2016). It is important to note that although zebrafish
349 reproduce easily in laboratory conditions, assessing inter-individual variability of
350 reproductive performances requires a large number of couples.

351 *No effect of irradiation was observed on adult reproduction performance*

352 For the testes dose rates, irradiation did not alter the reproductive capacity. Many
353 pollutants which contribute to oxidative stress are known to alter reproductive performance,
354 indicating that this physiological function may be sensitive to changes in the environment

355 (Faßbender et al. 2013; Newman et al. 2015; Simon et al. 2014; Wang et al. 2011).
356 Perturbation of reproduction was previously observed in other fish species. Woodhead (1977)
357 demonstrated that the total fecundity of guppies (*Poecilia reticulata*) was markedly reduced at
358 dose rates of 1.7, 4 and 12.7 mGy h^{-1} without drastic impacts on the mortality rate and
359 survival of offspring. Rackham et al. (1984) demonstrated that spermatogenesis of adult
360 butterfly splitfins (*Ameca splendens*) was disrupted (no production of sperm) after 7.3 mGy h^{-}
361 ^{1} of exposure over 52 days and that developing oocytes were less sensitive to the effects of
362 radiation than spermatogenesis. Knowles (2002) observed a significant decrease in adult
363 zebrafish in the mean number of eggs per spawn after 30 days at 7.4 mGy h^{-1}. Compared to
364 this study, the toxic modes of action seem to be strongly influenced by the biological model
365 and the experimental conditions of the dose rates. Under our experimental conditions, adult
366 zebrafish stage did not exhibit sensitivity.

367 *Drastic effects from irradiation on progeny survival were observed and transferred from*
368 *adult fish*

369 Drastic effects on progeny were only observed after high dose rate exposure and
370 certainly had a detrimental effect at the population level. After exposure of adults to 5 mGy h^{-}
371 ^{1} over 10 or 24 days (cumulative dose rates of 1.2 and 2.8 Gy, respectively), no effect on
372 progeny survival rate was observed until after 22 days. The range of these induced effects (5-
373 50 mgGy h^{-1}) is narrow and encourages us to more precisely determine the EDR_{10}. The non-
374 irradiated progeny of 27 d-F0 exposed to 53 mGy h^{-1} (34.3 Gy) previously showed a 100%
375 mortality rate occurring at the gastrulation stage (8 hpf) (Hurem et al. 2017a). In this study,
376 drastic effects appeared at a lower cumulative dose (12 Gy) for the same dose rate. These
377 results raise questions regarding the best criteria (cumulative dose *versus* dose rate) for
378 measuring gamma radiation exposure in the ERA.

379 The experimental design for the lowDR and highDR experiments did not allow the
380 identical cumulative dose to be obtained. It could potentially be beneficial to vary the duration
381 of exposure (1d at 50 mGy h^{-1}; 100d to 0.5 mGy h^{-1}) to compare the effects obtained at the
382 same cumulative dose.

383 Adverse effects seemed to be due to adult exposure since (i) irradiated and recovery
384 progeny showed identical effects compared to the control, and (ii) direct irradiation of
385 embryos at this high dose rate did not cause significant mortality as confirmed in this study
386 and by Simon et al, 2011. Vertebrate embryos are particularly sensitive to ionizing radiation,
387 due to a high rate of cell division and migration (Hu et al. 2016; Hurem et al. 2017a; Jarvis et
388 al. 2003; Rhee et al. 2012). The sensitivity of early stages (development and antioxidant
389 enzymes activities) has already been demonstrated; 6hpf-embryos were more sensitive than
390 12- and 24-hpf embryos to gamma irradiation (0.01-1 Gy) (Hu et al. 2016). However, we
391 demonstrated here that the sensitivity of the progeny to ionizing radiation was particularly
392 high when they originated from irradiated parents.

393 Parental effects observed in the highDR experiment were reversible, as they
394 disappeared when adults were placed in non-irradiated conditions for between 36 and 63 days.
395 These results suggest F0 recovery of reproductive capacity. The F0 adults zebrafish exposed
396 to 53 mGy h^{-1} during 27 days (31 Gy) and placed in non-irradiated conditions failed to
397 produce viable offspring 1.5 year after irradiation (Hurem et al. 2017a). The differences in
398 terms of experimental design and cumulative dose between the present study and that of
399 Hurem et al. (2017) encouraged us to elucidate the mechanisms behind the transfer from adult
400 to progeny and the F0 recovery induction over time.

401 The earliest stages of embryonic development rely on maternal products that are
402 generated during oogenesis and supplied to the egg. Yolk lipoprotein nutrients, vitamins,
403 hormones, mRNA transcripts, and DNA methylation statuses have been identified as markers
404 of egg quality. The damage to these maternal products could also explain the drastic effects
405 observed in the progeny. Note that the period of maternal control of embryonic development
406 varies among animals and could explain difference of ionizing radiation effects between
407 species (Abrams et al. 2009; Vastenhouw et al. 2019). Among transferred maternal products,
408 cortisol is essential for early development (Faught et al. 2016; Nesan et al. 2013; Nesan et al.
409 2016). Then, *de novo* synthesis of cortisol starts after hatching (48 hpf) (Nesan et Vijayan
410 2016). Finally, elevated cortisol levels in embryos leads to the same effects (mortality,
411 pericardial oedema and heart malformation) (Nesan et al. 2012) observed in this study.
412 Moreover, high levels of cortisol in females may directly impact estradiol production,
413 possibly affecting vitellogenin production and its incorporation into the oocyte, leading to
414 drastic effects in the progeny. In this study, we hypothesize that the high dose rates of gamma
415 irradiation could have led to an alteration of the cortisol deposition and its transfer to eggs,
416 which may have resulted in altered offspring phenotypes.

417 *ROS production and genotoxicity after irradiated and recovery conditions*

418 From potentially toxic mechanisms, the genotoxicity and ROS induction were assessed.
419 Gamma irradiation is known to induce ROS formation in zebrafish larvae (Gagnaire et al.
420 2015; Jarvis et Knowles 2003). In this study, ROS production compared to the control was
421 increased after 4 d of exposure of larvae originating from adults exposed during 10 and 24
422 days. Recovery larvae showed ROS production, without being irradiated. ROS production
423 was higher in F1 recovery larvae than in irradiated larvae (originating from F0-10d). Once
424 again, adult exposure led to high ROS production in larvae. This would seem to be a

425 consequence of an effect inherited from the parents. Results were similar to those observed in
426 other exposure conditions (dose rate, recovery duration) by Hurem et al, 2017.

427 As no effect was observed in 10 d-larvae, the oxidative stress induced by gamma
428 irradiation seems to be transitory. The activities of antioxidant enzymes are known to change
429 in a developmental stage-dependent manner. However, larval ROS production in 4 d larvae
430 was increased by gamma irradiation and persisted in F1 recovery condition. We hypothesized
431 that under our experimental conditions, irradiation of the progeny led to an induction of
432 protective agents that could counteract ROS production and re-establish a healthy cellular
433 redox balance. Antioxidant enzyme activities could be assessed in further experiments in 10
434 d-irradiated larvae to test this hypothesis.

435 Significant genotoxic effects were observed at 4 d-irradiated and non-irradiated progeny
436 coming from adults exposed in the lowDR experiment (0.5 and 5 mGy h^{-1}) during 24 days.
437 High inter-individual variability was however observed for the lowDR experiment, preventing
438 the highlighting of significant differences.

439 Genotoxicity has previously been demonstrated in eggs exposed to gamma rays in
440 chronic or acute experiments (Gagnaire et al. 2015; Praveen Kumar et al. 2017; Simon et al.
441 2011b) or to non-radioactive pollutants (Kosmehl et al. 2008; Vicquelin et al. 2011). In this
442 study, tail moment values were comparable to those measured in eggs/larvae exposed over 4 d
443 at 24 mGy h^{-1} (Gagnaire et al. 2015). Thus, these results suggest an increase in the intensity of
444 genotoxic effects due to parental irradiation and confirm the molecular effects inherited from
445 the parents. The genotoxic effects decreased at 10 d, suggesting a better efficiency of DNA
446 repair mechanisms at this larval stage, potentially related to a better efficiency of the

447 antioxidant system. It would be interesting to measure some of the DNA repair mechanisms
448 (as gamma H2AX activity) in order to confirm this hypothesis.

449 Irradiation of adults at high dose rates led to significant genotoxic effects on irradiated
450 and non-irradiated progeny as early as 1 d, unlike results obtained at lower dose rates.
451 However, the levels of genotoxic effects at 1 d remained lower than the ones measured at 4 d
452 in the lowDR experiment which did not lead to a high mortality rate. The comparison between
453 the different stages of development remains difficult and we have to take into account
454 possible differences in reparation mechanism induction between the different stages to explain
455 the relationship between mortality and genotoxicity effects. We suggest that the early live
456 stage could be more sensitive than the later life stage.

457 High irradiation exposure led to significant genotoxic effects on adult gonads. However,
458 the effects were lower than those measured after low uranium waterborne exposure (Simon et
459 al. 2018). Significant differences were also observed in the controls between males and
460 females, as previously observed in other studies (Simon et al. 2018). Moreover, irradiation
461 effects seemed more pronounced for males than for females. Further studies may be
462 completed by the determination of genotoxicity levels in zebrafish sperm, allowing a better
463 link with genotoxicity observed in the progeny, as proposed by Reinardy et al. (2013). Indeed,
464 during spermatogenesis, chromatin is processed and packaged and contact between DNA
465 and nuclear matrix is reorganized. Furthermore, the epigenetic pattern is also totally re-
466 modelled and transcription is stopped. All these events contribute to the control of embryo
467 development in the early-life stages (Herráez et al. 2017).

468

469 **CONCLUSION**

470 These results on fish reproductive performance after gamma irradiation provide
471 additional information for ERAs. Drastic effects on progeny and consequently on population
472 levels were observed at dose rates between 5 and 50 mGy h^{-1}, confirming the DCRL values.
473 However, it is important to note a difference in the sensitivity between the trout reference
474 organism and the *Danio rerio*, the model organism in our study. Moreover, this study
475 highlights that parental exposure leads to significant effects in the progeny at the molecular
476 level (ROS, genotoxicity), even at low dose rates (0.5 m Gy h^{-1}). The consequences of these
477 molecular effects will need to be studied following chronic exposure. The results encourage
478 us to take into account the consequences of transmitted generational (*i.e.* heritable) effects for
479 the determination of DCRL values. This study improves our understanding of the
480 consequences of multigenerational exposure conditions for a better radioprotection of aquatic
481 ecosystems. It confirms the difference in sensitivity of model species and identifies a narrow
482 range of occurrence of drastic effects. It also acts as an incentive to acquire new data from
483 multigenerational chronic exposure at low dose levels.

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633

634 **Figure legends**

635 Figure 1. Exposure duration, conditions and endpoints for lowDR and highDR experiments.
636 For each exposure condition, a control condition was included. For the lowDR experiment
637 (0.5, 5 mGy h^{-1}), adults were exposed over 24 days. Reproduction was assessed after 10 and
638 24 days of irradiation. The progeny were then placed in irradiated and non-irradiated (F1
639 Recovery) exposure conditions over 10 and 22 days. For the highDR experiment (50 mGy h^{-}
640 ^{1}), adults were exposed over 10 days. The progeny were then placed in irradiated and non-
641 irradiated (F1 Recovery) exposure conditions over 120 hours. 10d-irradiated adults were
642 placed in non-irradiated exposure (F0 Recovery) over 63 days. Reproduction was assessed
643 after 6, 36 and 63 days in F0 recovery conditions. The progeny were placed in non-irradiated
644 exposure conditions over 96 hours for each time point (6, 36 and 63 days).

645

646 Figure 2. Fecundity (number of egg per female) of F0 adults measured after 10 days of
647 exposure for both lowDR and highDR experiments (Treatment versus Control, *, Kruskal-
648 Wallis p=0.03). n=number of spawns.

649

650 Figure 3. Individual (points) and average (histograms, means ± sd) survival rates (%,) of F1
651 progeny at 24 and 48 h. F1 progeny came from adults irradiated during 10 days for all
652 experiments. F1 R: F1 recovery condition, *i.e* progeny spawned by irradiated adults and
653 placed in non-irradiated conditions.

654 For lowDR experiment (control, 0.5, 5, R0.5, R5 mGy h^{-1}), survival rate was calculated from
655 3 spawns (with 2 pseudo-replicates, 1 male+1 female).

656 For highDR experiment (control, 50, R50 mGy h^{-1}), survival rate was calculated from 6
657 spawns (1 male+1 female). (Kruskal-Wallis test: Treatment versus Control, a: p=0.024, b:
658 p=0.015, c: p=0.006, d: p=0.006; a>b>c>d).

659

660 Figure 4. Survival rate (%) of F1 progeny over time for lowDR experiment. Data are means ±
661 SD of two replicates of 80 eggs for 3 spawns per condition. (Kruskal-Wallis test: 5 versus
662 F1R, a: p=0.049, n=6).

663 A. F1 progeny coming from adults irradiated during 10 days. Immediately after spawning, F1
664 progeny was placed in irradiated and in non-irradiated (F1R Recovery) exposure conditions
665 during 22 days.

666 B. F1 progeny coming from adults irradiated during 24 days. Immediately after spawning, F1
667 progeny was placed in irradiated exposure conditions during 10 d.

668

669 Figure 5. Survival rate (%, n=6) of F1 progeny (3-4 hpf) over time. Progeny came from 10d-
670 irradiated adults for highDR experimentation. F1 Progeny were placed in irradiated and non-
671 irradiated (F1R, Recovery) exposure conditions over 120 h. (Kruskal-Wallis test: Treatment
672 versus Control, a: p=0.006, b: p=0.006, c: p=0.003, d: p=0.003; e=0.003, f: p=0.003).

673

674 Figure 6. Individual (points) and average (histograms, means ± SD) survival rates (%, n=4-6)
675 of F1 progeny after 24 and 48 h. Progeny came from adults irradiated during 10 days and then
676 placed in non-irradiated conditions (F0R, Recovery) during 63 d. (Kruskal Wallis test,

677 Treatment versus Control, a: p=0.015 n=12, b: p=0.0060 n=11, c: p=0.05 n=8, e: p=0.0058
678 n=11, f: p=0.05 n=11).

679

680 Figure 7. Body length (mm) and typical aspect of F1 progeny at 72 h. F1 progeny came from
681 adults exposed during 10d (Irr. 10d) and 10d followed by 6 days (F0 Irr. 10d+F0R 6d) and 36
682 days in non-irradiated (F0 Irr. 10d+F0R 36d) conditions. (Kruskal Wallis test, Treatment
683 versus Control, *, p<0.05, F0Irr. 10d p=0.002 n=20; 0.0049 n=20, F0 Irr. 10d+F0R 6d,
684 p=0.00007 n=33, F0 irr. 10d+F0R 36d, p=0.077, n=85).

685

686 Figure 8. ROS production measured in larval exposed during 4 d coming from F0 adults
687 exposed during 10 (A1) and 24d (A2) and in larval exposed during 10 d coming from F0
688 adults exposed during 24 d (B) of lowDR experiments. Nd: not determinate n=20 per
689 condition, Anova test *, p<0.05.

690 Figure 9. DNA damage (Tail moment) in F1 progeny after 1, 4 and 10d of exposure. Progeny
691 came from adults irradiated during 24d for lowDR experiment. Progeny (3-4 hpf) were placed
692 in irradiated and non-irradiated (F1R, Recovery) conditions. Kruskal Wallis test, *, p<0.05.

693

694 Figure 10. DNA damage (tail moment) in progeny (A: 1d, irradiated and non-irradiated
695 exposure (F1R, Recovery)) and in gonads of F0 adults (B: female, C: male) coming from
696 highDR experiment. Kruskal Wallis test: *, p<0.05.

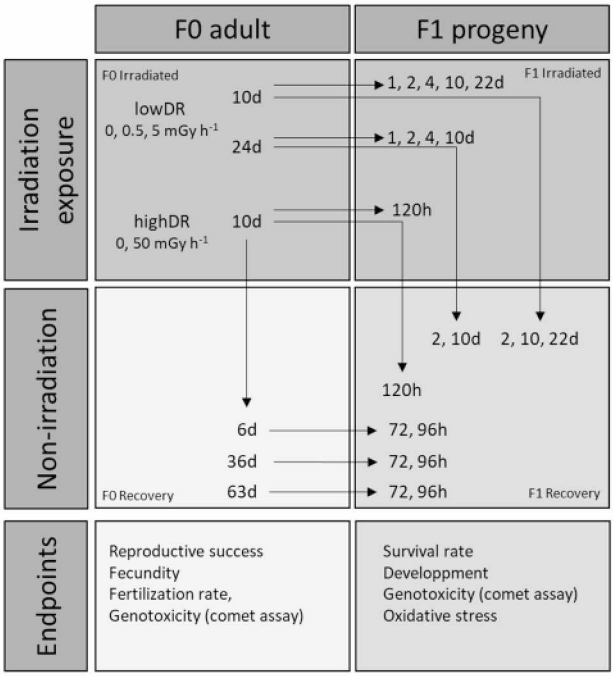

Figure 1. Exposure duration, conditions and endpoints for the lowDR and highDR experiments. For each exposure condition, a control condition was included. For the lowDR experiment (0.5, 5 mGy h-1), adults were exposed over 24 days. Reproduction was assessed after 10 and 24 days of irradiation. The progeny were then placed in irradiated and non-irradiated (F1 Recovery) exposure conditions over 10 and 22 days. For the highDR experiment (50 mGy h-1), adults were exposed over 10 days. The progeny were then placed in irradiated and non-irradiated (F1 Recovery) exposure conditions over 120 hours. 10 d-irradiated adults were placed in non-irradiated (F0 Recovery) exposure conditions over 63 days. Reproduction was assessed after 6, 36 and 63 days in F0 recovery conditions. The progeny were placed in non-irradiated exposure conditions over 96 hours for each time point (6, 36 and 63 days).

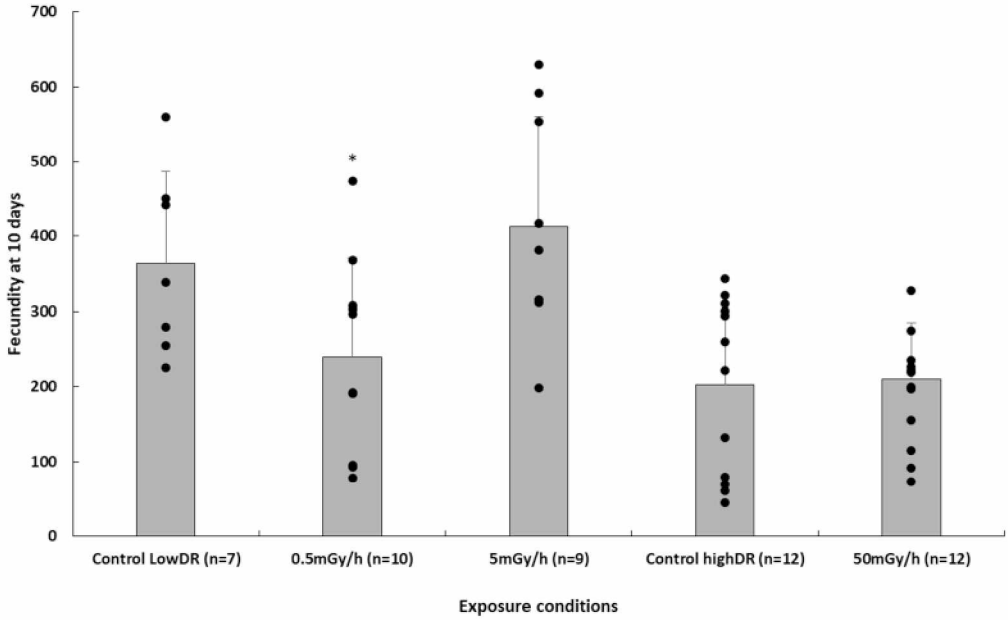

Figure 2. Fecundity (number of eggs per female) of F0 adults measured after 10 days of exposure for both the lowDR and highDR experiments (Kruskal-Wallis test: Treatment versus Control, * p=0.03). n=number of spawns.

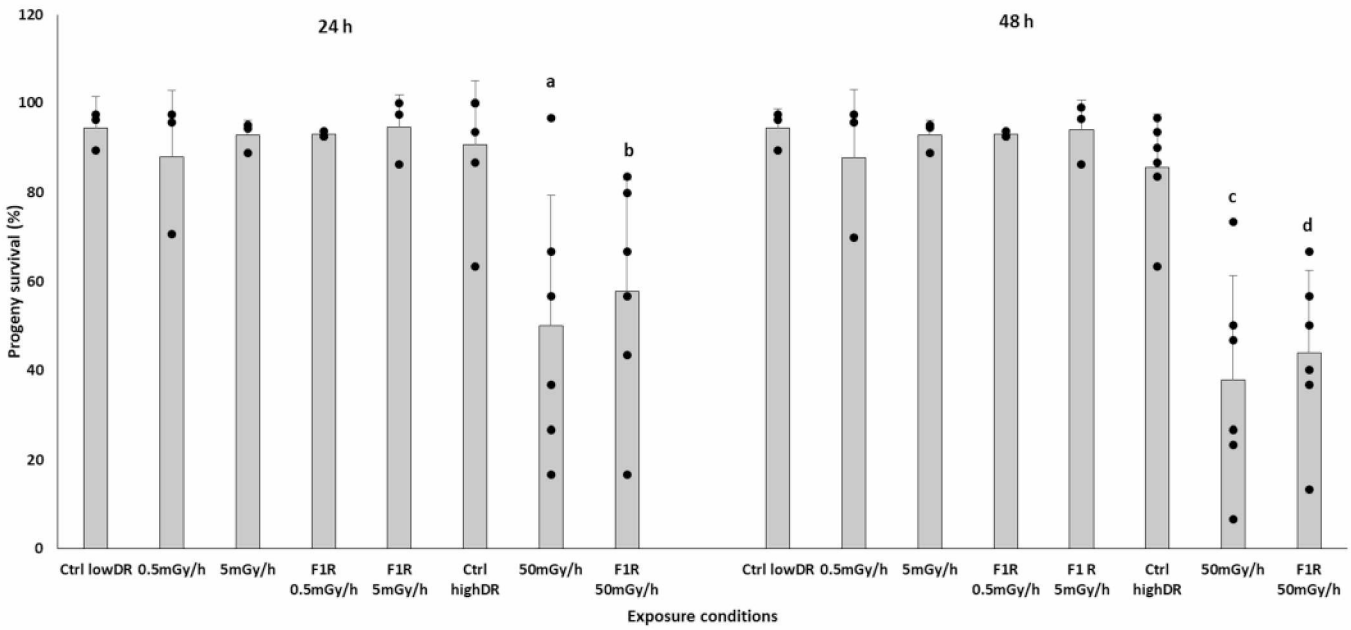

Figure 3. Individual (points) and average (histograms, means ± sd) survival rates (%, n=xx) of the F1 progeny at 24 and 48 h. F1 progeny came from adults irradiated during 10 days for all experiments. F1 R: F1 recovery condition, i.e. progeny spawned by irradiated adults and placed in non-irradiated conditions.

For the lowDR experiment (control, 0.5, 5, R0.5, R5 mGy h^{-1}), the survival rate was calculated from 3 spawns (with 2 pseudo-replicates, 1 male+1 female).

For the highDR experiment (control, 50, R50 mGy h^{-1}), the survival rate was calculated from 6 spawns (1 male+1 female). (Kruskal-Wallis test: Treatment versus Control, a: p=0.024, b: p=0.015, c: p=0.006, d: p=0.006; a>b>c>d).

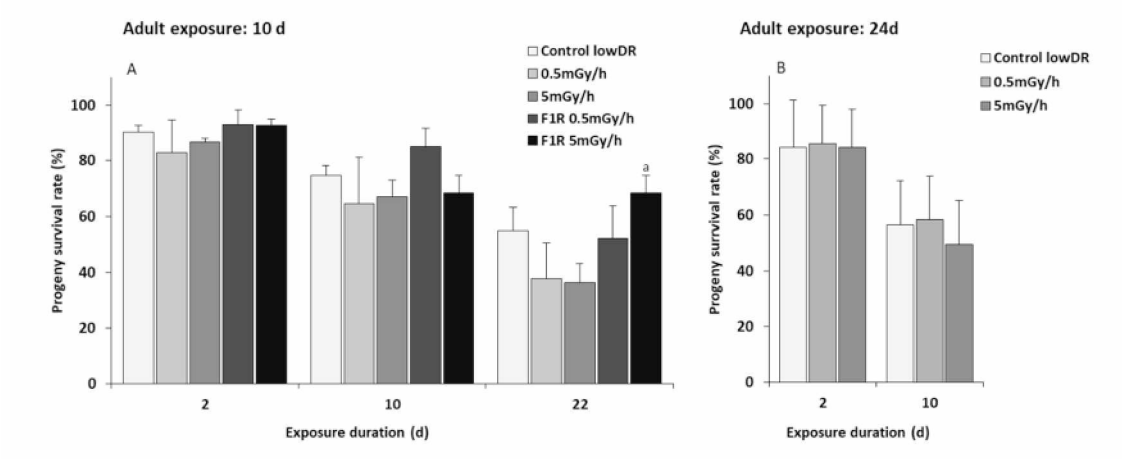

Figure 4. Survival rate (%) of F1 progeny over time for the lowDR experiment. Data are means ± SD of two replicates of 80 eggs for 3 spawns per condition. Kruskal-Wallis test: 5 versus F1R 5, a, n=6, p=0.049

A. F1 progeny coming from adults irradiated over 10 days. Immediately after spawning, the F1 progeny was placed in irradiated and in non-irradiated (F1R Recovery) exposure conditions over 22 days.

B. F1 progeny coming from adults irradiated over 24 days. Immediately after spawning, the F1 progeny was placed in irradiated exposure conditions over 10 d.

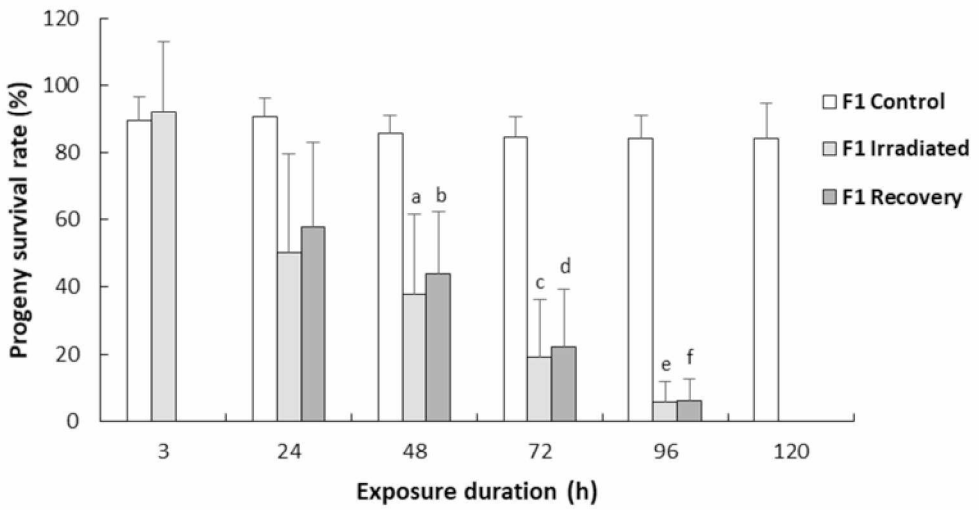

Figure 5. Survival rate (%) of 6 F1 progeny (3-4 hpf) over 120 h. The progeny came from 10 d-irradiated adults from the highDR experiment. The F1 Progeny were placed in irradiated and non-irradiated (F1R, Recovery) exposure conditions over 120 h. (Kruskal-Wallis test: Treatment versus Control, a: p=0.006, b: p=0.006, c: p=0.003, d: p=0.003; e:=0.003, f: p=0.003).

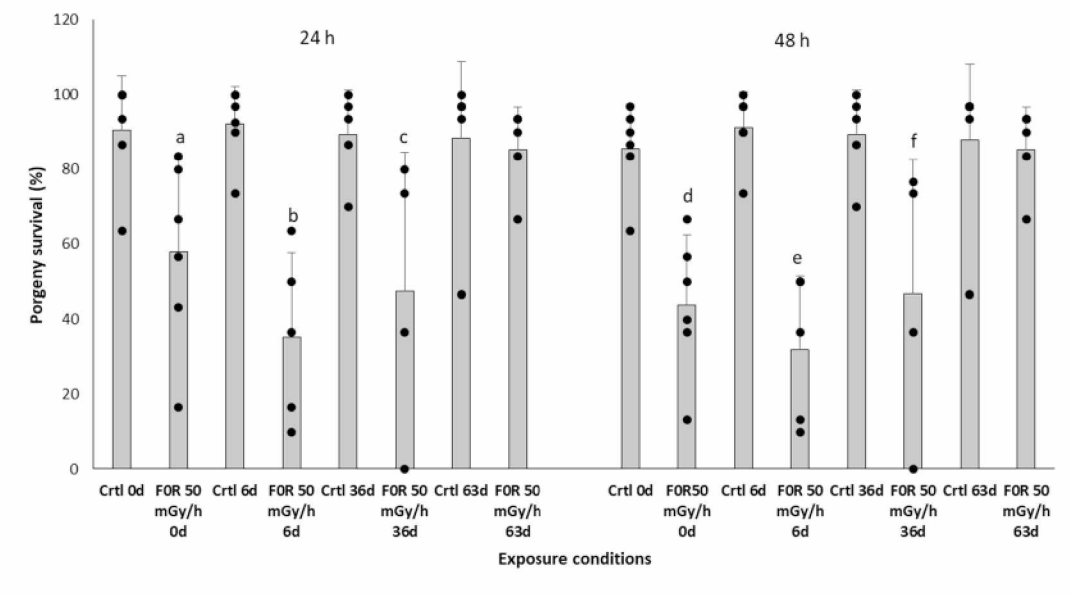

Figure 6. Individual (points) and average (histograms, means ± SD) survival rates (%, n=4-6) of F1 progeny after 24 and 48 h. The progeny came from adults irradiated over 10 days and then placed in non-irradiated conditions (F0R, Recovery) over 63 d. Kruskal Wallis test: Treatment versus Control a: p=0.015 n=12, b: p=0.0060 n=11, c: p=0.05 n=8, e: p=0.0058 n=11, f: p=0.05 n=11.

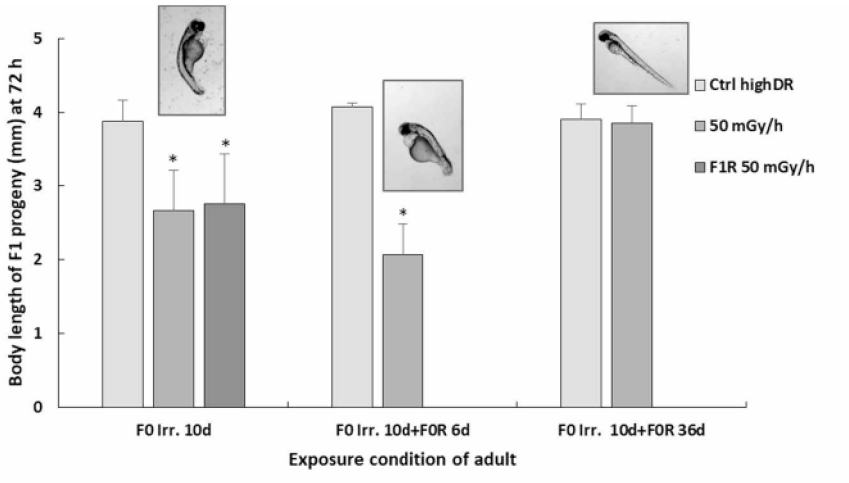

Figure 7. Body length (mm) and typical aspect of F1 progeny at 72 h. The F1 progeny came from adults exposed over 10 d (Irr. 10 d) and 10 d followed by 6 days (F0 Irr. 10d+F0R 6d) and 36 days in non-irradiated (F0 Irr. 10d+F0R 36d) conditions. Kruskal Wallis test, Treatment versus Control *, $p<0.05$, F0Irr. 10d $p=0.002$ $n=20$; 0.0049 $n=20$, F0 Irr. 10d+F0R 6d, $p=0.00007$ $n=33$, F0 irr. 10d+F0R 36d, $p=0.077$, $n=85$.

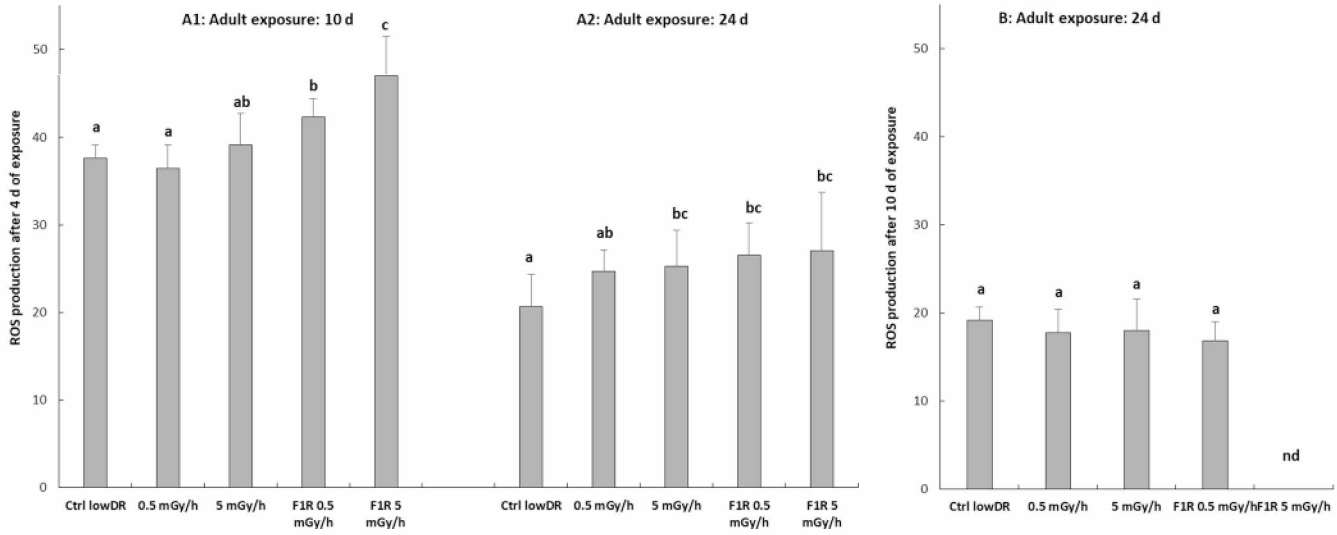

Figure 8. ROS production measured in larvae exposed over 4 d coming from F0 adults exposed over 10 (A1) and 24 d (A2) and in larvae exposed during 10 d coming from F0 adults exposed over 24 d (B) of lowDR experiment. Nd: not determinate n=20 per condition, Anova test *, $p<0.05$.

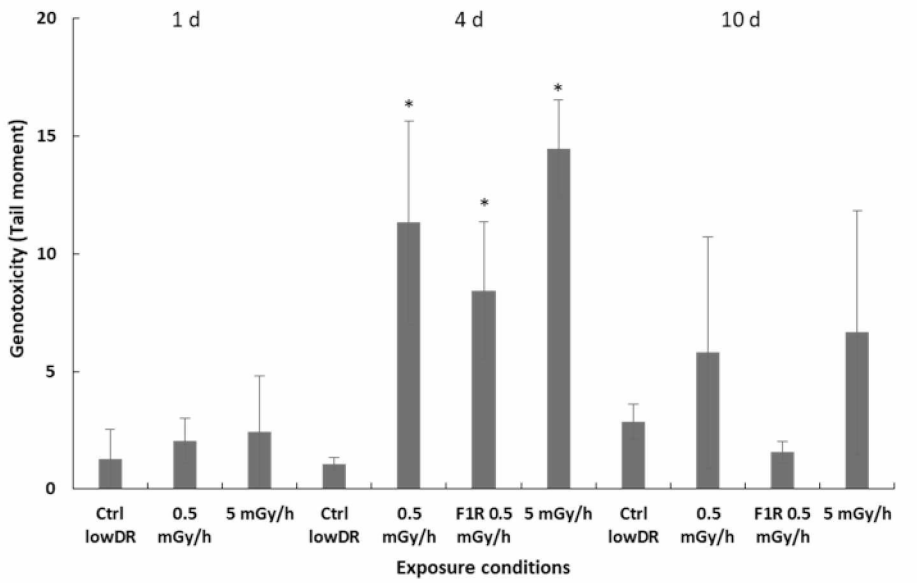

Figure 9. DNA damage (Tail moment) in the F1 progeny after 1, 4 and 10 d of exposure. The progeny came from adults irradiated over 24 d for the lowDR experiment. The progeny (3-4 hpf) were placed in irradiated and non-irradiated (F1R, Recovery) conditions. Kruskal Wallis test, Treatment versus Control *, $p<0.05$.

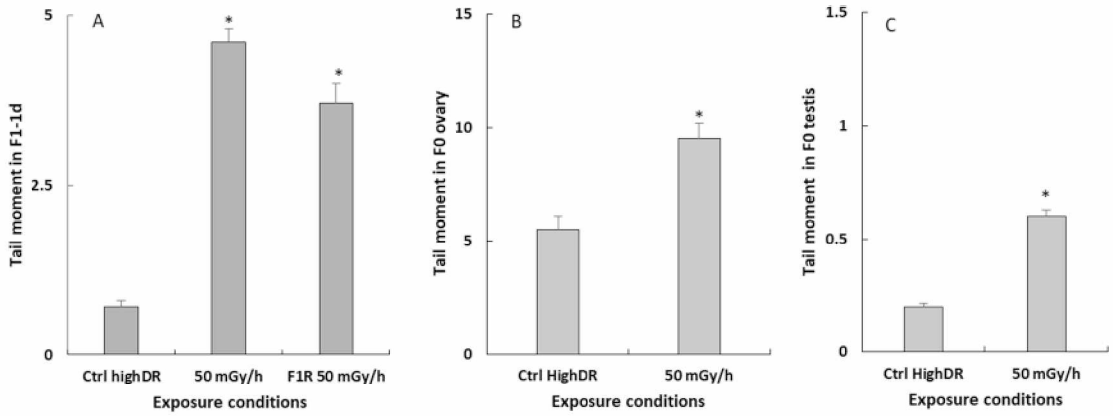

Figure 10. DNA damage (tail moment) in the progeny (A: 1 d, irradiated and non-irradiated exposure (F1R, Recovery)) and in gonads of F0 adults (B: female, C: male) coming from the highDR experiment. Kruskal Wallis test, Treatment versus Control *, $p<0.05$.