



HAL
open science

Multigenerational exposure to gamma radiation affects offspring differently over generations in Zebrafish

Noémie Guirandy, Beatrice Gagnaire, Virginie Camilleri, Isabelle Cavalie,
Fabien Pierron, Patrice Gonzalez, Olivier Simon

► **To cite this version:**

Noémie Guirandy, Beatrice Gagnaire, Virginie Camilleri, Isabelle Cavalie, Fabien Pierron, et al.. Multigenerational exposure to gamma radiation affects offspring differently over generations in Zebrafish. *Aquatic Toxicology*, 2022, 244, pp.106401. 10.1016/j.aquatox.2022.106101 . hal-03609780

HAL Id: hal-03609780

<https://hal.science/hal-03609780v1>

Submitted on 31 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Abstract**

2 Mutigenerational studies are now of great interest in ecotoxicology and previous
3 studies have shown the importance of conducting multigenerational studies when
4 assessing radiation toxicity in fish. In our study, the first objective was to study the
5 early life stages (embryo-larval stages) and critical functions such as reproduction
6 (which are generally studied in the context of ecological risk assessment (ERA)), in
7 order to assess its sensitivity. The second objective was to assess acquisition of
8 phenotypic effects at some life stages over generations.

9 To our knowledge, this was the first time that irradiation of zebrafish (0.05 and 5
10 mGy.h⁻¹) up to generation F2 was maintained with the following two exposure
11 conditions: (1) recovery, only F0 genitors were irradiated and the progeny were
12 placed in control condition, (2) irradiated condition, all generations were exposed.
13 Multigenerational irradiation affected F1 parental reproductive capacity (reproductive
14 success) mainly over the first reproductive cycle (104d) and larval survival rate.
15 Unexpected yet significant effects on sex ratio were observed in F1 progeny after
16 parental irradiation (mainly at 5 mGy.h⁻¹). These effects were observed for both
17 conditions -irradiated and recovery- suggesting transmitted effects from F0 genitor to
18 offspring. All studied life stages were affected by ionizing radiation (IR), suggesting
19 an alteration of vital physiological functions (reproduction and sexual determination).
20 Such results highlight the hypothesis that IR affects population dynamics. In addition,
21 the clear evidence of transmitted effects suggests worsening of effects at the
22 population scale over generations. This approach is closer to environmental
23 conditions to assess wild population fate, and thus highlights the importance of
24 multigenerational studies in support ERA of ionizing radiation in fish.

25 Keywords: multigenerational, mortality, sex ratio, zebrafish, irradiation, transmitted
26 effect

27 **Multigenerational exposure to gamma radiation affects**
28 **offspring differently over generations in Zebrafish**

29 Guirandy Noémie^{a*}, Gagnaire Béatrice^a, Camilleri Virginie^a, Cavalié Isabelle^a, Pierron
30 Fabien^b, Gonzalez Patrice^b, Simon Olivier^{a*}

31 ^a IRSN/PSE-ENV/SRTE/LECO, Centre de Cadarache-B.P. 3 – Bat 183 – 13115 St
32 Paul Lez Durance

33 ^b UMR EPOC CNRS 5805, Place du Docteur Bertrand Peyneau 33120 ARCACHON

34 *Corresponding author.

35 E-mail addresses: noemie.guirandy@irsn.fr

36

37 **1. Introduction**

38 Exposure to ionizing radiation (IR) is reported to induce a variety of biological effects
39 in fish (Anbumani and Mohankumar, 2012; Rhee et al., 2012). In most cases, the
40 effects after gamma irradiation are assessed at specific sensitive stages, such as the
41 embryo-larval stage, because they are considered to be most vulnerable to ionizing
42 radiation (Gagnaire et al., 2015; Hu et al., 2016; Lerebours et al., 2020; Pereira et al.,
43 2011; Praveen Kumar et al., 2017; Simon et al., 2011a). However, focusing on just
44 one sensitive stage of life is not enough to obtain a comprehensive understanding of
45 the effects of irradiation in fish (Guirandy et al., 2019; Hurem et al., 2017a; Hurem et
46 al., 2018). For example, at 50 to 53 mGy h⁻¹, F1 irradiated and non-irradiated

47 progeny from F0 irradiated zebrafish (*Danio rerio*) showed 100% mortality, providing
48 an evidence of transmitted effects from parents to progeny and thus the importance
49 of assessing multigeneration studies. These dose rates are not environmentally
50 relevant but contribute to the development of the dose-responses relationships
51 necessary for ERA. Further studies at lower dose rates also need to be considered.
52 At a lower dose rate (5 to 8.6 mGy h⁻¹), parental exposure led to significant effects at
53 the molecular levels in progeny, via multiple processes, such as epigenetic
54 mechanisms (Hurem et al., 2017b; Hurem et al., 2018; Kamstra et al., 2018).
55 Moreover, multigenerational studies assessed over an entire life span should be
56 done to represent at most the environmental conditions; indeed higher ecological
57 radiosensitivity was observed in Chernobyl wildlife (Hazard dose rate affecting 50%
58 of species at their 50% effect) compared to laboratory conditions.

59 The Chernobyl and Fukushima accidents released considerable amounts of
60 radionuclides into the environment and have provided a strong impetus to better
61 address the ecosystems affected by chronic exposure to gamma radiation
62 (Bréchnignac et al., 2016; Lerebours et al., 2016). However, traditional environmental
63 risk assessment (ERA) approaches do not generally consider multigenerational
64 exposure when predicting impacts on ecosystems. Therefore, classical ERA
65 benchmarks might not be adequate for assessing irradiation effects in wild fish
66 populations. Indeed, as for other pollutants, effects of IR are mainly assessed based
67 on classical endpoints (reproductive success, fecundity, fertility, survival) measured
68 at one stage of life, without considering the effects across multiple generations. The
69 lack of data on toxicity after multigenerational exposure have encouraged the
70 scientific community and policymakers to address laboratory multigenerational
71 studies (EC-TG N°27, 2011), which has led to the development of the “Test No.443:

72 Extended One-Generation Reproductive Toxicity Study” (OECD, 2018) on fish. .
73 However, multigeneration studies on IR are scarce but they demonstrated that
74 multigenerational exposure can lead to harmful effects and highlights new toxic
75 mechanisms (see Guirandy et al. 2019). These studies suggested that IR can induce
76 (i) epigenetic alterations, such as DNA methylation located on gene promoters and
77 enhancers, which can be inherited by future generations and (ii) altered
78 transcriptomes.

79 Beyond studied parameters, selecting an appropriate exposure dose or dose rate
80 exposure is also very important to increase the relevance of ecotoxicity datasets.
81 Frequently, irradiation effects on fish focus exclusively on acute and short-term
82 exposures (Hu et al., 2016; Pereira et al., 2011; Tsyusko et al., 2011), which is not
83 environmentally realistic and not suitable for assessing chronic effects. Low dose
84 rates must also be studied to complete the dose/response relationship and more
85 precisely represent the environmental conditions. A generic screening value of 10
86 $\mu\text{Gy h}^{-1}$ has been defined as protected dose rate for terrestrial and aquatic
87 ecosystems (Garnier-Laplace et al., 2010). Environmental protection by International
88 Commission on Radiological Protection (ICRP) referred to the Derived Consideration
89 Reference Levels (DCRL), corresponding to a dose rate band where deleterious
90 effects can appear. A possible reduction in reproductive success for dose rates
91 between 40 and 4000 $\mu\text{Gy h}^{-1}$ was retained for freshwater fish (Reference Animals
92 and Plant: trout) (ICRP, 2012).

93 In this study, we investigated whether multigenerational gamma irradiation (^{137}Cs) of
94 zebrafish (*Danio rerio*) can affect the reproductive performances of animals and
95 induce effects on F1 and F2 progenies (Guirandy et al., 2019; Hurem et al., 2017a;
96 Hurem et al., 2018; Kamstra et al., 2018). We hypothesized that IR can influence

97 reproductive performance across generations. Therefore, a multigenerational
98 experiment on fish covering three generations (F0 exposed at adult stage, F1 and
99 F2) was performed at a low dose rate of 0.05 mGy h^{-1} , close to the generic screening
100 value (0.01 mGy h^{-1} , (Garnier-Laplace et al. 2006)) and lower DCRL range. A second
101 dose rate of 5 mGy h^{-1} was studied to challenge the drastic effects on progeny
102 previously determined in experiments focusing on high irradiated parental exposure
103 (50 mGy h^{-1}) (Guirandy et al., 2019) and represents the upper dose rate of the DCRL.
104 We used domesticated zebrafish (*Danio rerio* AB strain), which develop rapidly, have
105 high fecundity, and are well described in the literature (Lawrence, 2007). *Danio rerio*
106 is often used in ecotoxicology, for the assessment of stress effects in general and
107 more recently for the characterization of multi-or transgenerational effects of
108 stressors (Baker et al., 2014; Lin et al., 2020; Pierron et al., 2021; Siegenthaler et al.,
109 2017; Simon et al., 2014). Moreover, zebrafish have been already used in studies
110 assessing the effects of ionizing radiation (Epperly et al., 2012; Gagnaire et al., 2015;
111 Houdigui et al., 2020; Hurem et al., 2017a; Hurem et al., 2018; Kamstra et al., 2018;
112 Kong et al., 2016; Simon et al., 2011a). This study was designed to answer several
113 questions: (i) How does gamma radiation impact different generations and how is this
114 impact transmitted across generations? (ii) What are the sensitive endpoints to
115 radiation at low doses or dose rate?

116 We reared F1 and F2 progenies from F0 adult irradiated for 30d. Two irradiation
117 conditions were defined: (i) irradiated (I) where each generation was irradiated over
118 time to observe any possible worsening of effects over the 2 generations and (ii)
119 recovery (R) where only the F0 adult was exposed, progenies were in non-irradiated
120 conditions to observe any possible parental transmission of effects. Adult
121 reproductive performances, progeny survival, and progeny development were

122 evaluated and compared to previous experiments performed at a high dose rate (50
123 mGy h⁻¹) (Guirandy et al., 2019).

124 **2. Materials and methods**

125 **2.1 Adult fish husbandry**

126 Project #20995 was authorized by the Institut de Radioprotection et de Sûreté
127 Nucléaire (IRSN) ethics committee no. 81 (EU 0520, C13-013-07) and complied with
128 French regulations on performing experiments on animals in application of directive
129 2010/63//UE relating to animal protection. The study was conducted on wild-type
130 zebrafish that were kept, reproduced, and irradiated in a zebrafish housing system
131 (Zebtec Tecniplast Stand Alone) with recirculating oxygenated freshwater. Adult fish
132 were acclimatized for 3 weeks to tap water + 20% demineralized water renewed daily
133 (Aquadem; pH = 7.4 ± 0.4, conductivity = 398 ± 12 µS cm⁻¹,
134 temperature = 28.4 ± 1.3 °C), with a 12:12-h light:dark cycle photoperiod. The fish
135 were fed *ab libitum* three times a day with GEMMA Wean (Skretting®).

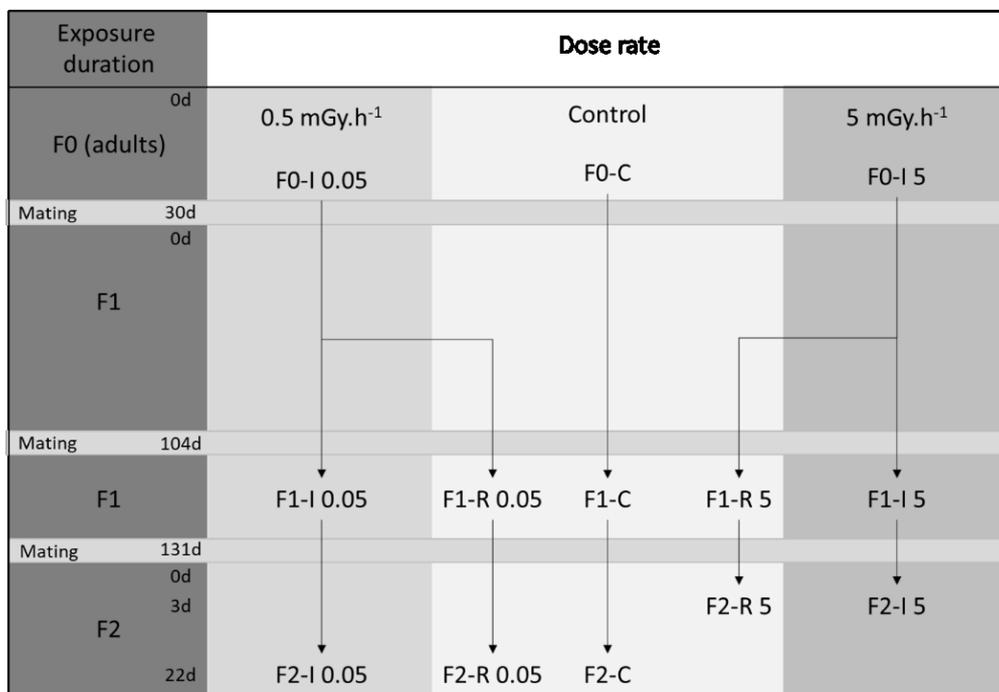
136 **2.2 Fish rearing**

137 The rearing method under controlled conditions was optimized based on different
138 tests performed before the experimentation (choices of food, cleaning of devices,
139 density of individuals, and water renewal for better survival), not presented here.
140 From 0 hpf to 20 days post-fertilization (dpf), progeny were kept in crystallizing glass
141 dishes (diameter 9 cm) in groups of 50 fish. The water used in crystallizing dishes
142 was the same as that used for F0 adult fish. Crystallizing dishes were kept in an
143 incubator (PANASONIC MIR-154), with nominal constant temperature of 28°C and a
144 12:12-h light:dark cycle photoperiod. From 15 dpf to 20 dpf, the water level in the
145 crystallizing dishes was raised by 0.5 centimeter every day. Crystallizing dishes were
146 cleaned daily. At 20 dpf, progeny were transferred to an aquarium (3.5L, Zebtec

147 Tecniplast Stand Alone) with a low water flow to avoid disrupting larvae locomotion.
 148 At adult age, water flow was increased to ensure appropriate water quality. From 7
 149 dpf to 50 dpf, larvae were fed twice a day with 24h-old *Artemia salina* Nauplii and
 150 once a day with Gemma Micro ZF (Skretting®). Past this age, fish were fed *ad libitum*
 151 three times a day with GEMMA Wean (Skretting®) with a food dispenser. The same
 152 protocol was used for breeding the F2 generation from F1 adults.

153 2.3 Adult and embryo exposure

154



155

156 *Figure 1: Experimental design and exposure conditions (duration (d) and dose rate (mGy h⁻¹) used for*
 157 *the multigenerational experiment. F0 adults were exposed over 30 days until reproduction. F1*
 158 *progenies were then placed in irradiated (F1-I0.05, F1-I5) and non-irradiated (recovery, F1-R0.05, F1-*
 159 *R5) exposure conditions over 131 days. Mating was performed at 104 and 131d. F2 progenies (F2-*
 160 *I0.05, F2-I5) from F1 irradiated adults were irradiated over 22 days. F2 progenies (recovery, F2-*
 161 *R0.05,) from F1 recovery adult were placed in non-irradiated exposure over 22 days. F2 progenies*
 162 *(recovery, F2-R5) from F1 recovery adults were placed in non-irradiated exposure conditions over 72*
 163 *hours. M: mating.*

164

165 Nominal dose rates were 60–80 nGy.h⁻¹ (control-C), 0.05 (I0.05) and 5 (I5) mGy.h⁻¹.

166 Gamma-rays were emitted from a ¹³⁷Cs source (444 GBq, 662 keV; IRSN, MICADO-

167 Lab platform). Dose rates were simulated using MCNP5 software and measured
168 using thermoluminescent dosimeters (Chiyoada Technologies), and the values
169 represented between 98% and 108 % of the nominal values. Control dishes were
170 kept in a separate room.

171 The population density of adult fish was 4 fish per liter. 30 couples of F0-adult fish per
172 condition (F0-C, F0-I0.05, F0-I5) were exposed over 30 days. Daily controls and
173 feeding were conducted as described in Guirandy et al. (2019). Adult mass (g, fresh
174 mass) was measured after dissecting the fish.

175 For F0 reproduction, F1 offspring were obtained from 15 spawning couples (30 fish, 1
176 female: 1 male) (i.e., replicates) per condition. Mating and viability were determined
177 as described in Guirandy et al. (2019). The embryos from 3 spawns per condition
178 were separated into 2 groups per spawn. The first one was kept in crystallizing
179 dishes with a density of 50 eggs per dish. The F1 embryos (F1-I0.05, F1-I5) from 3
180 spawns from across all 15 spawning couples were then positioned in an incubator
181 and irradiated at the same irradiation conditions as for F0. The second group (F1
182 recovery embryos (F1-R0.05; F1-R5)) was placed in non-irradiated conditions. F1
183 offspring were irradiated over 131 days. Several reproduction assays were initiated
184 between 104 and 131d to assess the reproductive capacity of all F1 fish. For the
185 reproductions performed at 104, 105, 111, 112d and 131d, the couples (1 female: 1
186 male) were formed from the observation of secondary sexual characteristics. During
187 the last reproductive cycle carried out at 131d, the sexes were checked during the
188 dissection of the fish.

189 For F1 reproduction, there were at least 8 spawning couples (1 female: 1 male) per
190 spawn and condition, except for F1-I5 (n=5). F2 offspring was then kept in the same
191 conditions as F1 embryos until 22 days (F2-I0.05 F2-R0.05) of exposure and until 72

192 hours (F1-I5 and F2-R5) of exposure. F2-I0.05 and F2-I5 came from the irradiated F1
193 generation. F2-R0.05 and F2-R5 came from the F1 recovery condition, where only
194 the F0 adults were irradiated. All fish from irradiated conditions were reproduced
195 under irradiation.

196 ***2.4 Ecologically representative endpoints for adults and for progeny***

197 Reproductive success (number of couples that spawned), the fecundity (number of
198 eggs per female) of adults (F0 and F1) and the quality of 4 hpf-eggs were assessed.

199 For each generation (F1 and F2), the progeny survival rate (%) was assessed daily
200 until the stage with no more death (22 dpf) for all conditions. Three technical
201 replicates of 50 eggs originating from 3 different spawns from across all 15 spawning
202 couples per condition were tested. Survival rate was presented for 3 stages: 4 dpf, 8
203 dpf and 22 dpf. They were chosen because 4 dpf is a commonly studied stage for
204 ecotoxicity bioassays; 8 dpf is a critical stage that corresponds to the beginning of the
205 self-feeding period without a yolk sac, and 22 dpf is the stage at which spontaneous
206 embryo mortalities seizes.

207 For the F1 generation, the male-female distribution was assessed based on
208 observable sexual characteristics for the remaining individuals (n: F1-C = 194; F1-
209 R0.05 = 124; F1-R5 = 136; F1-I0.05 = 127; F1-I5 = 119).

210 ***2.5 Theoretical population size***

211 Theoretical fish production was estimated for the F1 and F2 generations and for each
212 condition. At the beginning, there were 60 individuals per condition for the F0
213 generation. The total effective population was calculated with the product of number
214 of females, reproductive success rate, mean number of viable eggs and survival rate
215 of progeny (at 22 dpf or 72 hpf for F2) (Simon et al., 2011b). Error bars correspond to

216 incertitude from mean number of viable eggs number and survival rate: $\Delta TP = TP(\frac{\Delta \text{Mean number of eggs}}{\text{Mean number of eggs}} + \frac{\Delta \text{ survival rate}}{\text{survival rate}})$.

218 **2.6 Statistical analysis**

219 All data are presented as mean values \pm SD, with significance taken as $p < 0.05$. For
220 both F0 and F1 masses, conditions were compared using Anova with BoxCox
221 transformation when normality and homogeneity were not verified. Concerning data
222 relating to the F0 generation, conditions were compared using a GLM (Generalized
223 Linear Model, glm function in R). Poisson and binomial distributions were selected for
224 fecundity and reproductive success parameters respectively. Concerning data
225 relating to the F1 and F2 generations, conditions were compared using a GLMM
226 (Generalized Linear Mixed Model, glmm function in R). This model integrates the
227 non-independence of data. Our data are linked by spawn, corresponding to the three
228 spawns chosen for breeding embryos. The spawn was chosen as a “random” effect.
229 The binomial distribution was used to analyze all the parameters (reproductive
230 success, sex ratio, survival rate) expect for the fecundity parameter for which a
231 Poisson distribution was preferred. For reproductive success of F1 131 dpf, as the
232 dataset represented can be defined as quasi-complete separation, because of zero
233 values, the invariant Jeffreys prior method was used with the brglm package. The
234 analyses were performed using R software (R Core Team, 2013) with the following
235 packages : “tidyverse”, “here”, “knitr”, “lme4”, “MASS”, “car”.

236 **3. Results**

237 Regardless of treatment groups, no adult fish mortality was observed during the
238 experimental period.

239 **3.1 Cumulative doses after exposure conditions**

240 Measured dose rates (0.051 ± 0.002 and 5.15 ± 0.3 mGy h⁻¹, n=10) were close to
 241 nominal dose rates. For the F0 generation, cumulative doses, based on nominal dose
 242 rate, in adults ranged between 0.036 and 3.6 Gy (Table 1). Higher cumulative doses
 243 (0.16 and 15.7 Gy) were measured after 131d of F1 exposure.

244

245

246 *Table 1: Cumulative doses (Gy) calculated from the nominal dose rate for each condition (control,*
 247 *dose rate of 0.05 and 5 mGy h⁻¹ (I) and Recovery (R) and each generation (F0, F1, F2).*

Cumulative dose (Gy)				
Dose rate (mGy h ⁻¹)	F0		F1	
	30d	131d	72h	22d
Control	5.8E-05	2.5E-04	5.8E-06	4.2E-05
I 0.05	3.6E-02	1.6E-01	3.6E-03	2.6E-02
I 5	3.6E+00	1.6E+01	3.6E-01	-
R 0.05	-	2.5E-04	4.2E-05	4.2E-02
R 5	-	2.5E-04	5.8E-03	-

248

249 **3.2 Adult reproductive performances**

250 *Table 2: Mass (g, fresh weight), reproductive success (%) and fecundity of F0 and F1 adults after*
 251 *exposure to control (C), 0.05 and 5 mGy h⁻¹ (I) and Recovery (R) conditions * (p<0.05), ** (p<0.01), ****
 252 *(p<0.001).*

Condition	Age at reproduction (days)	Mass (fw, g)			Number of couples	Reproductive success (%)	Fecundity	Survival at 4 hpf (%)
		Male	Female	n				
F0 – C		0.59 ± 0.10	0.76 ± 0.21	28	30	57	362 ± 175	90.67 ± 12.48
F0 – I 0.05	270	0.53 ± 0.09	0.76 ± 0.21	27	30	47	247 ± 127*	99.90 ± 0.27
F0 – I 5		0.53 ± 0.06	0.64 ± 0.17	29	30	43	206 ± 128**	95.90 ± 15.40
F1 – C		-	-		20	75	178 ± 85	97.56 ± 5.72
F1 – I 0.05		-	-		32	53**	271 ± 152	97.30 ± 4.13
F1 – I 5	104	-	-		20	15***	243 ± 46	63.60 ± 13.28
F1 – R 0.05		-	-		20	35***	80 ± 95	81.63 ± 21.16
F1 – R 5		-	-		29	10***	179 ± 192	97.50 ± 2.20
F1 – C		0.43 ± 0.06	0.62 ± 0.17	8	15	80	399 ± 223	92.42 ± 21.62
F1 – I 0.05		0.40 ± 0.09	0.49 ± 0.12	8	15	67	298 ± 162	99.56 ± 0.53
F1 – I 5	131	0.52 ± 0.05	1.02 ± 0.41	5	5	60	245 ± 233	99.22 ± 0.77
F1 – R 0.05		0.47 ± 0.06	0.61 ± 0.14	8	15	100	283 ± 153	92.49 ± 24.68
F1 – R 5		0.55 ± 0.14	0.85 ± 0.17	8	12	33*	291 ± 204	81.37 ± 26.82

253

254 Adult genitors showed relatively homogeneous masses for both males and females
255 for generations F0 and F1 (Table 2). Concerning F0 reproduction, the reproductive
256 success (RS) of F0 - I5 adults was lower (43%), but not significantly different
257 compared to the control (57%). The egg quality expressed by the survival rate at 4
258 hpf was not impacted by gamma irradiation ($p.val>0.05$), however, fecundity was
259 impacted ($p.val<0.05$). Concerning F1 reproduction, differences were observed
260 between the two reproductive tests. For the first reproduction test (104d), the RS for
261 all irradiated conditions were lower compared to control conditions, in particular for
262 F1-I5 (15%) and F1-R5 (10%), which were significantly different from the control
263 values. For the second reproduction test (131d), F1 - I0.05 and F1 - R0.05 showed a
264 RS similar to F1-C, but with a high reproduction for F1-R0.05 (100%). RS in the F1-
265 R5 group (33%, $n=12$) was significantly different from control values (80%, $n=15$),
266 and there was a decreasing trend compared to the F1-I5 (60%, $n = 5$) group,
267 although this was not statistically significant. Also, for this generation, no significant
268 difference was observed regarding the fecundity among treatment groups.

269 Egg quality (survival at 4 hpf) was greater than 90% and was not impacted by
270 exposure to IR; however a decreasing trend was observed for the first reproductive
271 cycle of F1-I5 (63.6%), F1-R0.05 (81.6%) and for the second reproductive cycle of
272 F1-R5 (81.4%). High variability among couples was observed, which reduced
273 statistical power and is likely the reason for the lack of significant effects. Gamma
274 irradiation could lead to a decrease in RS (from -10% to -60%). Note that for the 2nd
275 reproductive cycle, females from F1-I5 were as few as 5 because only 5 females
276 were able to reproduce from the tested adult fish.

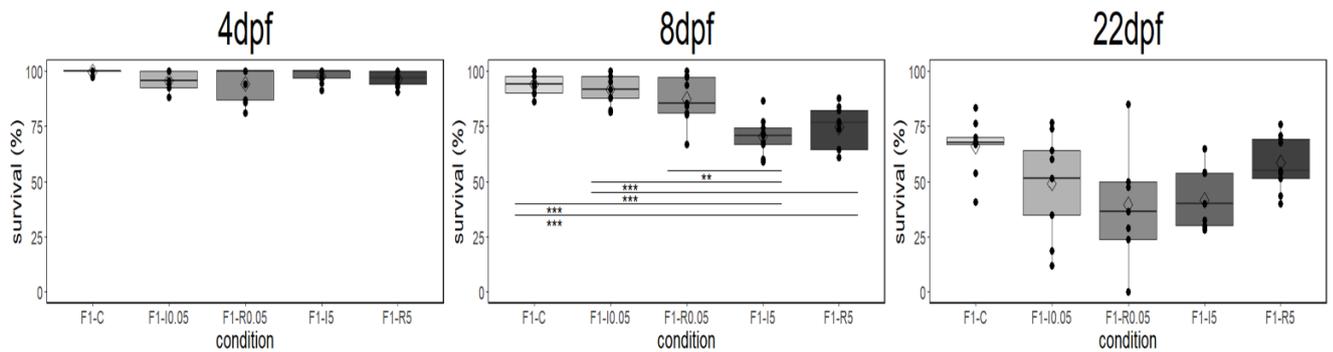
277 No relationship was observed between egg quality, fecundity and RS.

278 **3.3 Larval stage**

279

280 **3.3.1 For F1**

281



282

283 *Figure 2: Box-plot of survival rate of progeny (%) over time for the 1st generation (F1) at 4, 8 and 22*
284 *dpf after control (C), dose rate exposures to 0.05 and 5 mGy h⁻¹(I) and recovery (R) conditions. The*
285 *boxplot represent the 25th and the 75th percentile with the median indicated by blackline. Dots*
286 *represent individual data. Means are indicated as empty lozenge. For each condition, n = 9, **
287 *(p<0.05), ** (p<0.01), *** (p<0.001).*

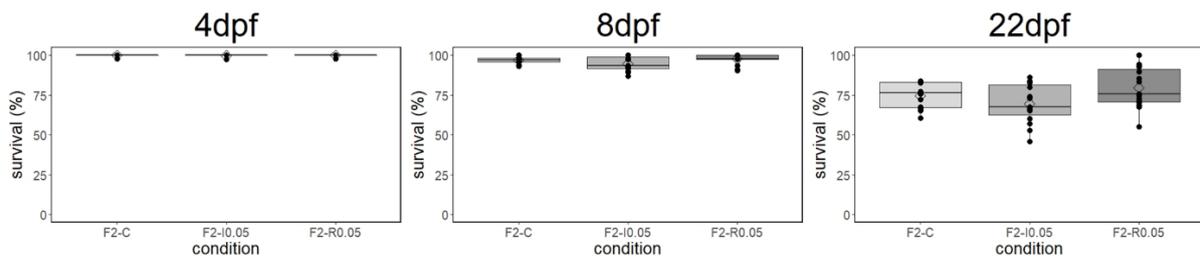
288 At 4 dpf, the average percentage of survival observed was high and between 94.2
289 and 99.7% for all exposure conditions (Figure 2). No significant difference was
290 observed among treatment groups when comparing more than 2 conditions.
291 However, a high individual variability was observed for F1-R0.05 group (SD = 7.66).
292 At 8 dpf, the average percentage of survival in F1-I5 (70.4%) and F1-R5 (74.6%)
293 conditions was significantly lower than the controls. A trend towards increased
294 survival rate was observed for F1-R5 vs F1-I5. At 22 dpf, the average percentage of
295 survival observed was 66.0, 49.2, 39.6, 41.5 and 58.5% for F1-C, F1-I0.05, F1-
296 R0.05, F1-I5 and F1-R5, respectively and showed high individual variability (SD
297 between 12.4 and 31.2), which may explain the lack of statistical differences among
298 conditions. The highest variability for F1-R0.05 occurred in two replicates. Finally, the

299 decrease in survival rate over time was more significant for irradiated conditions than
300 control conditions. No further mortalities were observed after 22dpf until 131dpf.

301 3.3.2 For F2

302

303



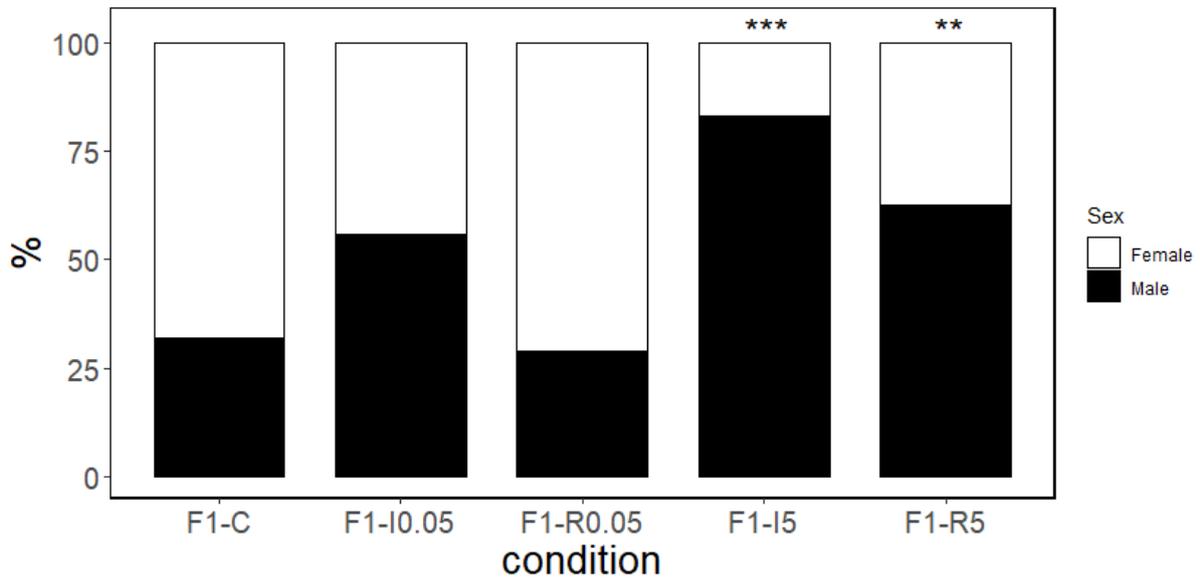
304

305 *Figure 3: Box-plot of survival rate of progeny (%) over time for the 2nd generation (F2) at 4, 8 and 22*
306 *dpf after control (C), dose rate exposures to 0.05 and 5 mGy h⁻¹ (I) and recovery (R) conditions. The*
307 *boxplot represent the 25th and the 75th percentile with the median indicated by blackline. Dots*
308 *represent individual data. Means are indicated as empty lozenge. For each condition, n = 15, **
309 *(p<0.05), ** (p<0.01), *** (p<0.001).*

310 Concerning F2 generation survival (reproduction at 131d), the individual range of
311 variability was low for the first stages (Figure 3) but increased at 22 dpf; however
312 variability was clearly lower than for the F1 generation. No significant difference of
313 survival rate was observed among F1-I0.05, F1-R0.05 and control values. The effect
314 of irradiation in the F2 generation appeared less noticeable than that measured in the
315 F1 generation. The survival rate (%) was only assessed at 3 dpf for F2-I5 (53.3%,
316 n=3 breeding pairs) and was lower than that measured for the first reproductive cycle
317 (data not shown).

318 **3.4 Adult stage of F1 generation**

319 **3.4.1 Sex ratio**



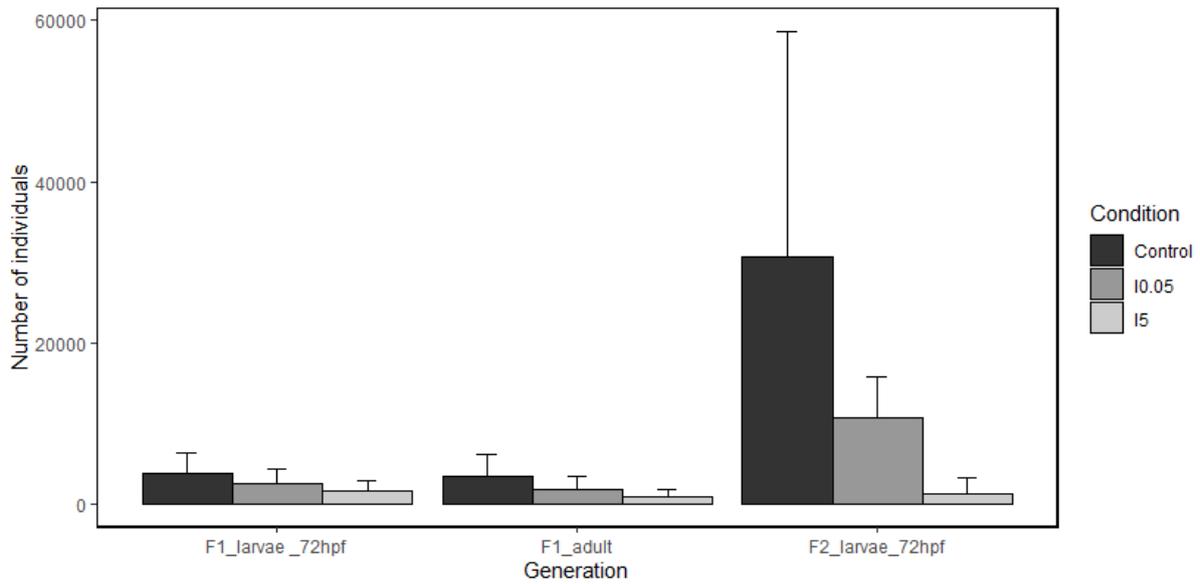
320

321 *Figure 4: Female and male F1 adult distribution (%) a in control (C), 0.05 and 5 mGyh⁻¹ irradiated (I)*
322 *and in recovery (R) conditions. at 131 days. Total number of adult fish; F1-C = 194; F1-R0.05 = 124;*
323 *F1-R5 = 136; F1-I0.05 = 127; F1-I5 = 119. * (p<0.05), ** (p<0.01), *** (p<0.001).*

324 Significant differences were observed concerning the male and female distribution for
325 the F1 adults (Figure 4). Greatest female (%) disruption was for F1-I5 (17%, 20
326 females) and F1-R5 (37%) compared to F1-C (68%). No difference was observed
327 between F1-I5 and F1-R5, whereas a significant difference was seen between F1-
328 I0.05 and F1-R0.05. The percentage of females was higher for F1-R0.05 than for F1-
329 I0.05 and showed a comparable pattern to F1-C (68%).

330

331 **3.5 Population size**



332

333 *Figure 5: Estimation of population of F1 and F2 generations after irradiation exposures (Control*
 334 *conditions (C), to 0.05 and 5 mGy h⁻¹ (I). At the beginning, 60 fish were present (sex ratio = 1 male: 1*
 335 *female) per condition for the F0 generation. The total effective population was calculated with the*
 336 *product of number of females, reproductive success rate, fecundity (mean number of viable eggs) and*
 337 *the survival rate of progeny (at 3 and 22 dpf for F1 and 3 dpf for F2).*

338 For the F1 generation, the average population of F1-C (3493 individuals) was higher
 339 than F1-I0.05 (x2), and F1-I5 (x3.8) (Figure 5). At 72 hpf, data were identical to 22
 340 dpf for F1 – C (22 dpf: 3493; 72 hpf: 3715). Thus, it was decided to use survival at 72
 341 hpf to include a condition with 5 mGy h⁻¹ irradiation in the description of variation in
 342 population. For the F2 generation, the theoretical population of F2-C (30701
 343 individuals) was much higher than that of F2-I0.05 (x2.9) and F2-I5 (x26.3). Note that
 344 for F2-I5, RS was done only for females previously able to reproduce. The most
 345 severe effects were observed for condition I5 while a slight improvement was
 346 observed for condition I0.05 between the two generations. By projecting the values of
 347 endpoints measured in the second generation, up to the third generation, the number

348 of adult fish is 12 times lower for condition I0.05 and more than 600 times lower for
349 condition I5 compared to control conditions.

350 **4. Discussion**

351 After gamma irradiation exposure to 0.05 and 5 mGy h⁻¹ over two generations,
352 adverse effects were shown in F1 generation. All life stages were affected with early
353 mortality, poor RS in first reproduction and altered male biased sex ratio.

354 **4.1 Multigenerational effects**

355 Significant decreases in reproductive success (RS) (30% for F1-I0.05, 80% for F1-I5
356 compared to F1-control conditions) were observed for the first F1 reproductive cycle
357 (104d) compared to the F0 reproductive cycle (18% for F0-I0.05, 25% for F0-I5,
358 compared to F0-control). Only 15% of all F1-I5 couples was able to reproduce with
359 low egg quality (64±13%).

360 Reproduction was tested at 104d, an age greater than the age of sexual maturity
361 (90d) in the Danio ((Lawrence, 2007). Since all fish were under the same
362 reproductive conditions, the hypothesis was that irradiation during the entire oocytes
363 development cycle of F1 adults would impair reproduction. This is also justified by the
364 fact that between the first reproduction (104 days), for which all mature oocytes have
365 been expelled, and the second reproduction (131 days), for which the mature II
366 oocytes were irradiated only for 27 days, an improvement of RS was observed;
367 however, RS of irradiated F1 adults was still lower than the control values (Table
368 2)..Effects on reproductive capacities have already been observed for other
369 contaminants such as endocrine pollutants (Li et al., 2019), which are widely studied,
370 when compared to IR. Since nuclear power plants are widely used for energy
371 production, it appears necessary to study the impacts of radionuclide releases more
372 extensively. As effects are observed even from low doses, with a decrease in

373 reproductive success, the question of the consequences on population dynamics
374 may arise. For zebrafish, the irradiation time for developing mature oocytes is short
375 (27 days) and this results in an insignificant decrease in RS. In contrast, after 104
376 days of irradiation of mature oocytes, a significant decrease in RS was observed. For
377 wild fish species, which often have a long maturation time with just one reproduction
378 per year, a decrease in reproductive success could have severe consequences.

379 A significant effect on F1-I5 (70%) and F1-R5 (75%) survival rates was observed at 8
380 dpf compared to control conditions (90%), a key stage of development (Geffroy and
381 Simon, 2013; Lawrence, 2007) whereas no effect was observed when embryos from
382 unexposed parents were chronically irradiated at this dose rate (Gagnaire et al.,
383 2015; Houdigui et al., 2020; Hurem et al., 2017b; Simon et al., 2011a). The same
384 was found for other species of fish (Guppy embryos (up to 8.4 Gy) or mosquito fish
385 (12-50 Gy)) (ICPR, 2008). Mortality for *D. rerio* larvae was only observed after acute
386 and short-term irradiation (10 Gy, 1.16 Gy/min) (Pereira et al., 2011; Praveen Kumar
387 et al., 2017). At 8 dpf, the survival rate at the lower dose rate was identical to the
388 control group, suggesting a tolerance to lower dose rates. We hypothesize that no
389 significant effect was observed at 5 mGy h⁻¹ and 22 dpf because of high individual
390 variability among F1 individual eggs. Number of individuals and technical replicates
391 were sufficient but eggs originated from only 3 genitors, and some genitors produced
392 eggs of poor quality. Moderate effects were still observed even when progeny was not
393 irradiated. However, these results were different to those observed previously,
394 although a comparable trend with a decrease of survival rate had been observed at
395 22d (5 mGy h⁻¹, (Guirandy et al., 2019)). We hypothesized that this difference may be
396 a function of differences in sensitivity of the batches of fish (background of different
397 genitors, age, strains) used for the different experiments. For the second generation,

398 F2-I5 showed a low survival rate (53%; 3dpf) obtained only from 3 spawns. Additional
399 investigations will be necessary to confirm the trend in survival rate observed for F1-
400 I0.05 and F1-R0.05.

401 Results indicated that parental exposure had consequences on the survival rate of
402 progeny, as also observed for other biological models (Buisset-Goussen et al., 2014;
403 Gilbin et al., 2008; Parisot et al., 2015). Parental exposure could be considered as a
404 critical window of sensitivity in F1 development (Ivy et al., 2017) since effects were
405 observed at the phenotypic scale but also at the molecular scale after genitor
406 irradiation (Hurem et al., 2017a).

407 For ERA, life stage specific sensitivity must be considered alongside exposure
408 conditions to define threshold values. Here, effects at 5 mGy.h⁻¹ were significant but
409 much less than at 50 mGy.h⁻¹ (Guirandy et al., 2019). So, it would be worthwhile to
410 conduct experiments with dose rates between 5 and 50 mGy h⁻¹ to confirm this gap in
411 survival rate after parental irradiation.

412 No mortality was observed from 22 to 104/131 dpf, suggesting that direct or indirect
413 (transgenerational) effects of IR only affected early development stages. The results
414 confirm the sensitivity of early embro-larval stage of *D. rerio* after parental exposure.

415 Unexpected effects were observed at the highest dose rate (5 mGy h⁻¹). Selection of
416 couples based on secondary sexual characteristics had been proven to be difficult,
417 and when sex was determined after dissection of adults, the sex ratio was
418 significantly biased in favor of males, with 4 and 1.8 times lower numbers of females
419 for F1-I5 and for F1-R5, respectively. Irradiation could affect sex differentiation as
420 previously observed after exposure to hypoxia, high temperature and pollutants in
421 zebrafish (Brion et al., 2004; Pierron et al., 2021; Valdivieso et al., 2020; Wang et al.,
422 2011). Exposure to heat in fish is usually known to increase the number of males.

423 The zebrafish housing system (Zebtec Tecniplast Stand Alone) used recirculating
424 oxygenated freshwater that prevents hypoxia and temperature variation. Responses
425 to environmental changes can be mediated by epigenetic mechanisms as discussed
426 by Pierron et al. (2021) and Valdivieso et al. (2020). Significant effects were obtained
427 without progeny irradiation (F1-R5) but were higher when the progeny were also
428 irradiated (F1-I5). F0 adult irradiation affected the non-exposed F1 generation. The
429 sex ratio expected for zebrafish reared under control laboratory conditions is
430 theoretically 50:50 (female to male) or with a small predominance of males (40:60)
431 (Santos et al., 2017; Simon et al., 2014). This was not the case in our study, where
432 females were predominant (68%). Previous studies have shown that sex ratio was
433 linked with maintenance conditions, such as temperature, density and nutrition
434 (Pierron et al., 2021). In our case, control and recovery group were maintained under
435 the same conditions. Therefore, the significant difference in the sex ratio between
436 these two conditions can be attributed to the irradiation and not to the bias of the sex
437 ratio of our population.

438 In gonochoric species such as zebrafish, the gonads are "ovary-like" before genetic
439 determination and then differentiation into male or female gonads. Since zebrafish
440 sex is determined by genetic factors (genetic sex-determination (GSD)) and
441 irradiation targets the genome, larval mortality and disruption of sex ratio effects at 5
442 mGy h⁻¹ could be induced by genetic mechanisms.

443 Moreover, zebrafish sex determination could be influenced by environmental factors
444 (environmental sex-determination (ESD)) such as hypoxia, temperature, EDCs,
445 population density, and food (Santos et al., 2017; Valdivieso et al., 2020). The
446 precise mechanisms of these environmental factors are not understood, but studies
447 suggest that the endocrine stress-axis could play a critical role. In medaka, a GSD

448 species, temperature induces masculinization through an increase in cortisol
449 (Fernandino et al., 2012; Hayashi et al., 2010). It has also been shown that cortisol
450 was able to induce the masculinization of both behavioral and morphological traits of
451 female *Gambusia affinis* (Geffroy and Bardonnnet, 2016). Moreover, many studies
452 have pointed out that cortisol is able to alter the production of gonadal steroids,
453 because the enzymes involved in their synthesis (11- β HSD) are also involved in
454 producing/inactivating glucocorticoid. Cortisol suppresses the brain-pituitary-gonadal
455 (BPG) axis in females, leading to lower pituitary gonadotropin content, reduced
456 plasma sex steroid levels, and decreased gonadal weight (Tovo-Neto et al., 2020). In
457 trout (*Salmo gairdneri*), adding cortisol to water during sexual differentiation triggered
458 testis differentiation and leads to a male-biased population. Moreover, cortisol
459 inhibited aromatase production, which in turn resulted in male-biased offspring.
460 Aromatase expression and/or activities in zebrafish have been shown to be disrupted
461 by environmental pollutants (Hinfrey et al., 2018). Since RI at high dose rates
462 produce a stressful environment, we hypothesized that RI can stimulate cortisol
463 production, which is the stress hormone produced under stressed environment.
464 These observations strongly encourage us to measure the level of cortisol.

465 Cumulative effects on RS, sex ratio and survival rate observed at 5 mGy h⁻¹ led to a
466 significant decrease in effective population (x26.3). Note that the impact on the
467 population was calculated from F1 effects observed during the second reproductive
468 cycle. Greater effects on the F1 generation observed during the first reproductive
469 cycle led to a more significant decrease in effective population. Effects on the F2
470 population only affected the survival rate (72hpf), which decreased compared to F1
471 and should be confirmed with more replicates and life stages since we stopped the
472 experiment at 72hpf. Beyond the decrease in effective population, disturbing the sex

473 ratio can have major consequences for mating competition and success, and on the
474 behavior of territorial males and female aggressiveness. The sex ratio for the R5
475 condition was also significantly biased, leading to an imbalance in the population. At
476 0.05 mGy h⁻¹, an insignificant decreasing trend in the population size was observed.

477 Multigenerational exposure was also used to assess the worsening of effects over
478 generations. The decrease in RS compared to control values was observed over the
479 two generations in comparable proportions (I0.05: 0.82; I5: 0.75, table 2) between F0
480 and the second F1 reproduction. The worsening of the effects of irradiation on this
481 parameter for both dose rates was observed when F1 reproduced for the first time.
482 Concerning the fitness of the larval stage, the F2-I5 survival rate was low and only
483 evaluated at 3 dpf. Effects appears earlier during development for F2 generation
484 compared to F1 generation, highlighting a worsening effect. Since these results were
485 preliminary, effects on F2 survival should be confirmed . For the lower dose rate, no
486 effect on F2-I0.05 survival rate was observed, rather indicating an improvement
487 despite irradiation. Finally, the disruption of the sex ratio confirms the worsening of
488 the effects between the 2 generations. Future studies should determine sex in the F2
489 generation to confirm this effect.

490 The survival rate showed slight improvement under the recovery conditions. Note that
491 recovery fish were only exposed during the F0 generation. Although no significant
492 difference was observed, the survival rate was slightly better for F1-R5 than for F1-I5.
493 This observation could not be confirmed for 0.05 mGy h⁻¹ because of the high levels
494 of variability for the F1-R0.05 condition. It would be worth assessing potential repair
495 mechanisms at the molecular scale between these two types of exposure scenarios.
496 Reversible effects could also be explained by epigenetic mechanisms. However,
497 some epigenetic marks appeared to persist over multiple generations at 8.7 mGy h⁻¹

498 (Kamstra et al., 2018). Molecular effects, due to their high sensitivity, could persist
499 longer while phenotypic effects are more prone to recover over time.

500 ***4.2 Irradiation-impacted stages of life or physiological functions***

501 The recovery condition was studied to show differences between irradiated and non-
502 irradiated progeny born from irradiated couples. It should be kept in mind that the
503 irradiation of early stages from parents not exposed to these dose rates does not
504 affect the survival of the embryos (Gagnaire et al., 2015; Guirandy et al., 2019;
505 Houdigui et al., 2020; Hurem et al., 2017a; Simon et al., 2011a). Parental exposure
506 led to great or moderate effects on survival rate at 50 (Guirandy et al., 2019) and 5
507 mGy h⁻¹ (this study) of F1 progeny, respectively. This confirms the sensitivity of this
508 biological stage. However, as survival rate and sex ratio were also affected in the
509 recovery condition, we can hypothesize that the irradiation mainly affects late
510 gametogenesis in adults, where it leads to effects on progeny after exposure to 5 and
511 50 mGy h⁻¹. Stage III of oogenesis is the process of vitellogenesis, in which the
512 oocyte begins to incorporate Vtg and several maternally-transferable compounds
513 (Faught and Vijayan, 2018). The latter are involved in various key processes such as
514 cortisol and thyroid hormone regulation, immunological responses, endocrine stress
515 axis development, epigenetic (*de novo* DNA methyltransferases) and post-
516 transcriptional (miRNA pathway components and specific miRNAs) regulation of
517 gene expression (Vera-Chang et al., 2019). The alteration of these maternally-
518 transferable compounds, as observed for different molecular markers (Guirandy et
519 al., 2019; Hu et al., 2016; Hurem et al., 2017a; Kamstra et al., 2018) after parental
520 irradiation can have late repercussions on zebrafish development.

521 **5. Conclusion**

522 This paper investigated the effects of gamma irradiation on *Danio rerio* after
523 multigenerational exposure to two dose rates (0.05 and 5 mGy h⁻¹). It needs to be
524 acknowledged that the Danio model cannot represent all fish species, especially with
525 regard to effects on reproductive processes. This study completed a previous study
526 that focused on the effects of a high dose rate (50 mGy h⁻¹) on the same biological
527 model. The results obtained provide comprehensive insights into the diversity of the
528 responses to gamma irradiation dose rates. Moreover, this study answered many
529 questions concerning irradiation methods that should be taken into account in future
530 studies: (i) Multigeneration exposure shows The survival rate showed slight
531 improvement under the recovery conditions that each generation was impacted
532 differently or was not impacted at the phenotypic scale. Irradiation (0.05 and 5 mGy
533 h⁻¹) may affect different life stages (adult: reproductive success, sex ratio and larval
534 mortality). These findings emphasize an impact on some physiological functions
535 (gametogenesis, sexual determination). Such effects can also affect population
536 dynamics. Further experimentation is required to confirm these results. Moreover,
537 due to the diversity of responses from one dose to another, it is necessary to study a
538 wide panel of doses.

539 (ii) Multigenerational exposure makes it possible to acquire data on the reproductive
540 capacities of adults exposed throughout their lifespan and on the fitness of the
541 embryo-larval stages. These data, with high ecological value, can be used to roughly
542 assess population dynamics and the worsening (or not) of the effects. Performing
543 assays that assess effects of IR at different biological stages of *Danio rerio*
544 separately could provide less realistic information than a single multigenerational
545 assay.

546 Since wild populations are suspected to be more sensitive to radiation than
547 laboratory populations, this could partly be explained by worsening effects after
548 exposure over generations although model species have a lower polymorphism than
549 wild species.

550

551 ***Acknowledgements***

552 We would like to acknowledge the technical staff and Cantabella Elsa, of the two
553 laboratories IRSN/PSE-ENV/SRTE/LECO and IRSN/PSE-ENV/SRTE/LR2T for the
554 use of the MICADO'lab platform and dissection days. We would like to acknowledge
555 the Agency Walker Services - Traduction for English language assistance and
556 Shramana Dey for final proofreading and English corrections.

557 ***Funding sources***

558 Funding: This work was supported by the Institut de Radioprotection et de Sûreté
559 Nucléaire (IRSN).

560

561 **References**

562 Anbumani, S., Mohankumar, M.N., 2012. Gamma radiation induced micronuclei and
563 erythrocyte cellular abnormalities in the fish *Catla catla*. *Aquatic Toxicology* 122-123, 125-
564 132.
565 Baker, T.R., Peterson, R.E., Heideman, W., 2014. Using zebrafish as a model system for
566 studying the transgenerational effects of dioxin. *Toxicological Sciences* 138, 403-411.
567 Bréchignac, F., Oughton, D., Mays, C., Barnthouse, L., Beasley, J.C., Bonisoli-Alquati, A.,
568 Bradshaw, C., Brown, J., Dray, S., Geras'kin, S., Glenn, T., Higley, K., Ishida, K., Kapustka, L.,
569 Kautsky, U., Kuhne, W., Lynch, M., Mappes, T., Mihok, S., Møller, A.P., Mothersill, C.,
570 Mousseau, T.A., Otaki, J., Pryakhin, E., Rhodes, O.E., Jr., Salbu, B., Strand, P., Tsukada, H.,
571 2016. Addressing ecological effects of radiation on populations and ecosystems to improve
572 protection of the environment against radiation: Agreed statements from a Consensus
573 Symposium. *Journal of Environmental Radioactivity* 158-159, 21-29.
574 Brion, F., Tyler, C.R., Palazzi, X., Laillet, B., Porcher, J.M., Garric, J., Flammarion, P., 2004.
575 Impacts of 17β -estradiol, including environmentally relevant concentrations, on
576 reproduction after exposure during embryo-larval-, juvenile- and adult-life stages in
577 zebrafish (*Danio rerio*). *Aquatic Toxicology* 68, 193-217.

578 Buisset-Goussen, A., Goussen, B., Della-Vedova, C., Galas, S., Adam-Guillermin, C., Lecomte-
579 Pradines, C., 2014. Effects of chronic gamma irradiation: A multigenerational study using
580 *Caenorhabditis elegans*. *Journal of Environmental Radioactivity* 137, 190-197.
581 EC-TG N°27, 2011. European commission, technical Guidance For Deriving Environmental
582 Quality Standards. Guidance Document N°27.
583 Epperly, M.W., Bahary, N., Quader, M., DeWald, V., Greenberger, J.S., 2012. The zebrafish-
584 *Danio rerio* - Is a useful model for measuring the effects of small-molecule mitigators of late
585 effects of ionizing irradiation. *In Vivo* 26, 889-897.
586 Faught, E., Vijayan, M.M., 2018. Maternal stress and fish reproduction: The role of cortisol
587 revisited. *Fish and Fisheries* 19, 1016-1030.
588 Fernandino, J.I., Hattori, R.S., Kishii, A., Strüssmann, C.A., Somoza, G.M., 2012. The cortisol
589 and androgen pathways cross talk in high temperature-induced masculinization: The 11 β -
590 hydroxysteroid dehydrogenase as a key enzyme. *Endocrinology* 153, 6003-6011.
591 Gagnaire, B., Cavalié, I., Pereira, S., Floriani, M., Dubourg, N., Camilleri, V., Adam-Guillermin,
592 C., 2015. External gamma irradiation-induced effects in early-life stages of zebrafish, *Danio*
593 *rerio*. *Aquatic Toxicology* 169, 69-78.
594 Garnier-Laplace, J., Della-Vedova, C., Andersson, P., Copplestone, D., Cailes, C., Beresford,
595 N.A., Howard, B.J., Howe, P., Whitehouse, P., 2010. A multi-criteria weight of evidence
596 approach for deriving ecological benchmarks for radioactive substances. *Journal of*
597 *Radiological Protection* 30, 215-233.
598 Geffroy, B., Bardonnat, A., 2016. Sex differentiation and sex determination in eels:
599 Consequences for management. *Fish and Fisheries* 17, 375-398.
600 Geffroy, B., Simon, O., 2013. Effects of a *Spirulina platensis*-based diet on zebrafish female
601 reproductive performance and larval survival rate. *Cybiurn* 37, 31-38.
602 Gilbin, R., Alonzo, F., Garnier-Laplace, J., 2008. Effects of chronic external gamma irradiation
603 on growth and reproductive success of *Daphnia magna*. *Journal of Environmental*
604 *Radioactivity* 99, 134-145.
605 Guirandy, N., Gagnaire, B., Frelon, S., Munch, T., Dubourg, N., Camilleri, V., Cavalié, I.,
606 Floriani, M., Arcanjo, C., Murat El Houdigui, S., Armant, O., Adam-Guillermin, C., Gonzalez, P.,
607 Simon, O., 2019. Adverse effects induced by chronic gamma irradiation in progeny of adult
608 fish not affecting parental reproductive performance. *Environmental Toxicology and*
609 *Chemistry* 38, 2556-2567.
610 Hayashi, Y., Kobira, H., Yamaguchi, T., Shiraishi, E., Yazawa, T., Hirai, T., Kamei, Y., Kitano, T.,
611 2010. High temperature causes masculinization of genetically female medaka by elevation of
612 cortisol. *Molecular Reproduction and Development* 77, 679-686.
613 Hinfrey, N., Sohm, F., Caulier, M., Chadili, E., Piccini, B., Torchy, C., Porcher, J.M., Guiguen, Y.,
614 Brion, F., 2018. Dynamic and differential expression of the gonadal aromatase during the
615 process of sexual differentiation in a novel transgenic *cyp19a1a*-eGFP zebrafish line. *General*
616 *and Comparative Endocrinology* 261, 179-189.
617 Houdigui, S.M.E., Adam-Guillermin, C., Armant, O., 2020. Ionising radiation induces
618 promoter dna hypomethylation and perturbs transcriptional activity of genes involved in
619 morphogenesis during gastrulation in zebrafish. *International Journal of Molecular Sciences*
620 21.
621 Hu, M., Hu, N., Ding, D., Zhao, W., Feng, Y., Zhang, H., Li, G., Wang, Y., 2016. Developmental
622 toxicity and oxidative stress induced by gamma irradiation in zebrafish embryos. *Radiation*
623 *and Environmental Biophysics* 55, 441-450.

624 Hurem, S., Gomes, T., Brede, D.A., Lindbo Hansen, E., Mutoloki, S., Fernandez, C., Mothersill,
625 C., Salbu, B., Kassaye, Y.A., Olsen, A.K., Oughton, D., Aleström, P., Lyche, J.L., 2017a. Parental
626 gamma irradiation induces reprotoxic effects accompanied by genomic instability in
627 zebrafish (*Danio rerio*) embryos. *Environmental Research* 159, 564-578.

628 Hurem, S., Martín, L.M., Brede, D.A., Skjerve, E., Nourizadeh-Lillabadi, R., Lind, O.C.,
629 Christensen, T., Berg, V., Teien, H.C., Salbu, B., Oughton, D.H., Aleström, P., Lyche, J.L.,
630 2017b. Dose-dependent effects of gamma radiation on the early zebrafish development and
631 gene expression. *PLoS ONE* 12.

632 Hurem, S., Martín, L.M., Lindeman, L., Brede, D.A., Salbu, B., Lyche, J.L., Aleström, P.,
633 Kamstra, J.H., 2018. Parental exposure to gamma radiation causes progressively altered
634 transcriptomes linked to adverse effects in zebrafish offspring. *Environmental Pollution* 234,
635 855-863.

636 ICPR, 2008. Environmental Protection-the concept and use of reference animals and plants.
637 ICPR publication 108.

638 ICRP, 2012. Protection of the Environment under different exposure conditions. *Annals of*
639 *the ICRP*

640 Ivy, C.M., Robertson, C.E., Bernier, N.J., 2017. Acute embryonic anoxia exposure favours the
641 development of a dominant and aggressive phenotype in adult zebrafish. *Proceedings of the*
642 *Royal Society B: Biological Sciences* 284.

643 Kamstra, J.H., Hurem, S., Martin, L.M., Lindeman, L.C., Legler, J., Oughton, D., Salbu, B.,
644 Brede, D.A., Lyche, J.L., Aleström, P., 2018. Ionizing radiation induces transgenerational
645 effects of DNA methylation in zebrafish. *Scientific Reports* 8.

646 Kong, E.Y., Cheng, S.H., Yu, K.N., 2016. Zebrafish as an in vivo model to assess epigenetic
647 effects of ionizing radiation. *International Journal of Molecular Sciences* 17.

648 Lawrence, C., 2007. The husbandry of zebrafish (*Danio rerio*): A review. *Aquaculture* 269, 1-
649 20.

650 Lerebours, A., Nagorskaya, L., Gudkov, D., Smith, J., 2016. Effect of radiation on the health of
651 fish from Chernobyl, COMET workshop 30 years after the Chernobyl accident what do we
652 know about the effects of radiation on the environment ?

653 Lerebours, A., Robson, S., Sharpe, C., Smith, J.T., 2020. Subtle effects of radiation on embryo
654 development of the 3-spined stickleback. *Chemosphere* 248.

655 Li, S., Wu, Q., Sun, Q., Coffin, S., Gui, W., Zhu, G., 2019. Parental exposure to tebuconazole
656 causes thyroid endocrine disruption in zebrafish and developmental toxicity in offspring.
657 *Aquatic Toxicology* 211, 116-123.

658 Lin, W., Guo, H., Wang, L., Zhang, D., Wu, X., Li, L., Qiu, Y., Yang, L., Li, D., Tang, R., 2020.
659 Parental Transfer of Microcystin-LR-Induced Innate Immune Dysfunction of Zebrafish: A
660 Cross-Generational Study. *Environmental Science and Technology* 54, 1014-1023.

661 OECD, 2018. Test No. 443: Extended One-Generation Reproductive Toxicity Study. OECD
662 guidelines for the testing of chemical, section 4. .

663 Parisot, F., Bourdineaud, J.P., Plaire, D., Adam-Guillermin, C., Alonzo, F., 2015. DNA
664 alterations and effects on growth and reproduction in *Daphnia magna* during chronic
665 exposure to gamma radiation over three successive generations. *Aquatic Toxicology* 163, 27-
666 36.

667 Pereira, S., Bourrachot, S., Cavalie, I., Plaire, D., Dutilleul, M., Gilbin, R., Adam-Guillermin, C.,
668 2011. Genotoxicity of acute and chronic gamma-irradiation on zebrafish cells and
669 consequences for embryo development. *Environmental Toxicology and Chemistry* 30, 2831-
670 2837.

671 Pierron, F., Lorioux, S., Héroin, D., Daffe, G., Etcheverria, B., Cachot, J., Morin, B., Dufour, S.,
672 Gonzalez, P., 2021. Transgenerational epigenetic sex determination: Environment
673 experienced by female fish affects offspring sex ratio. *Environmental Pollution* 277.
674 Praveen Kumar, M.K., Shyama, S.K., Kashif, S., Dubey, S.K., Avelyno, D., Sonaye, B.H., Kadam
675 Samit, B., Chaubey, R.C., 2017. Effects of gamma radiation on the early developmental
676 stages of Zebrafish (*Danio rerio*). *Ecotoxicology and Environmental Safety* 142, 95-101.
677 R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation
678 for Statistical Computing, Vienna, Austria
679 Rhee, J.S., Kim, B.M., Kang, C.M., Lee, Y.M., Lee, J.S., 2012. Gamma irradiation-induced
680 oxidative stress and developmental impairment in the hermaphroditic fish, *Kryptolebias*
681 *marmoratus* embryo. *Environmental Toxicology and Chemistry* 31, 1745-1753.
682 Santos, D., Luzio, A., Coimbra, A.M., 2017. Zebrafish sex differentiation and gonad
683 development: A review on the impact of environmental factors. *Aquatic Toxicology* 191, 141-
684 163.
685 Siegenthaler, P.F., Zhao, Y., Zhang, K., Fent, K., 2017. Reproductive and transcriptional effects
686 of the antiandrogenic progestin chlormadinone acetate in zebrafish (*Danio rerio*).
687 *Environmental Pollution* 223, 346-356.
688 Simon, O., Floc'h, E., Geffroy, B., Frelon, S., 2014. Exploring ecotoxicological fish bioassay for
689 the evaluation of uranium reprotoxicity. *Environmental Toxicology and Chemistry* 33, 1817-
690 1824.
691 Simon, O., Massarin, S., Coppin, F., Hinton, T.G., Gilbin, R., 2011a. Investigating the
692 embryo/larval toxic and genotoxic effects of γ irradiation on zebrafish eggs. *Journal of*
693 *Environmental Radioactivity* 102, 1039-1044.
694 Simon, O., Mottin, E., Geffroy, B., Hinton, T., 2011b. Effects of dietary uranium on
695 reproductive endpoints-fecundity, survival, reproductive success-of the fish *Danio rerio*.
696 *Environmental Toxicology and Chemistry* 30, 220-225.
697 Tovo-Neto, A., Martinez, E.R.M., Melo, A.G., Doretto, L.B., Butzge, A.J., Rodrigues, M.S.,
698 Nakajima, R.T., Habibi, H.R., Nóbrega, R.H., 2020. Cortisol directly stimulates spermatogonial
699 differentiation, meiosis, and spermiogenesis in Zebrafish (*Danio rerio*) testicular explants.
700 *Biomolecules* 10.
701 Tsyusko, O., Glenn, T., Yi, Y., Joice, G., Jones, K., Aizawa, K., Coughlin, D., Zimbrick, J., Hinton,
702 T., 2011. Differential genetic responses to ionizing irradiation in individual families of
703 Japanese medaka, *Oryzias latipes*. *Mutation Research - Genetic Toxicology and*
704 *Environmental Mutagenesis* 718, 18-23.
705 Valdivieso, A., Ribas, L., Monleón-Getino, A., Orbán, L., Piferrer, F., 2020. Exposure of
706 zebrafish to elevated temperature induces sex ratio shifts and alterations in the testicular
707 epigenome of unexposed offspring. *Environmental Research* 186.
708 Vera-Chang, M.N., Moon, T.W., Trudeau, V.L., 2019. Ancestral fluoxetine exposure sensitizes
709 zebrafish to venlafaxine-induced reductions in cortisol and spawning. *Endocrinology* 160,
710 2137-2142.
711 Wang, M., Chen, J., Lin, K., Chen, Y., Hu, W., Tanguay, R.L., Huang, C., Dong, Q., 2011. Chronic
712 zebrafish PFOS exposure alters sex ratio and maternal related effects in F1 offspring.
713 *Environmental toxicology and chemistry* 30, 2073-2080.

714

715