Subtle effects of radiation on embryo development of the 3-spined stickleback

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Abstract

The Chernobyl and Fukushima nuclear power plant (NPP) accidents that occurred in 1986 and 2011 respectively have led to many years of chronic radiation exposure of wildlife. However, controversies remain on the dose threshold above which an impact on animal health occurs. Fish have been highly exposed immediately after both accidents in freshwater systems around Chernobyl and in freshwater and marine systems around Fukushima. The dose levels decreased during the years after the accidents, however, little is known about the effects of environmental low doses of radiation on fish health. The present laboratory study assesses the effects of an environmentally relevant dose range of radiation (0.1, 1 and 10 mGy/day) on early life stages of the 3-spined stickleback, *Gasterosteus aculeatus*.

The cardiac physiology and developmental features (head width, diameter, area) of high exposed embryos (10 mGy/day) showed no significant change when compared to controls. Embryos exposed to the medium and high dose were slower to hatch than the controls (between 166 and 195 hours post-fertilization). After 10 days of exposure (at 240 hours post-fertilization), larvae exposed to the high dose displayed comparable growth to controls. High-throughput sequence analysis of transcriptional changes at this time point revealed no significant changes in gene regulation compared to controls regardless of exposure conditions. Our results suggest that exposure of fish embryos to environmental radiation elicits subtle delays in hatching times, but does not impair the overall growth and physiology, nor the gene expression patterns in the recently hatched larvae.

Introduction

Serious nuclear accidents at both the Chernobyl Nuclear Power Plant (NPP) in April 1986 and the Fukushima NPP in March 2011 led to high levels of radiation exposure to wildlife. After the Chernobyl accident, the dose to fish found in the cooling pond was estimated at 10 mGy/day[1] and then rapidly declined due to the decrease of short life radionuclides and sedimentation processes[2].

Three decades after the Chernobyl NPP accident, the main radionuclides of concern are ^{90}Sr (a β emitter) and ^{137}Cs (a β and γ emitter) due to their long radioactive half-life (28 and 30 years respectively). Transuranium radioelements are also of concern due to their long radioactive half-life and high energetic alpha particle emission. However, their contribution to the total dose to fish at Chernobyl is very low[3]. In a highly contaminated lake called Glubokoye, located near the Chernobyl NPP, the total dose rate to perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) 30 years after the accident was up to 16 and 14 μGy/h respectively (less than 0.4 mGy/day)[3].

^{32}P is a radioisotope of 14.26 days half-life that emits β particles of high energy (1.7 MeV) whose track length is a few millimetres in water. A β-particle directly ionizes molecules by the removal of an electron, whereas α particles (and X-ray) ionize molecules by generating a series of fast electrons (effectively β particles) after first hitting molecules, therefore, the use of a β-emitter is relevant to assess the effect of environmental radiation. In order to assess the effects of ionising radiation exposure on fish embryos under laboratory conditions, ^{32}P was selected due to its short half-life minimising the radioactive waste and its high energy β particles.

^{32}P is used in research laboratories, medical procedures and industry that may lead to discharge into freshwater systems. The Krasnoyarsk Mining and Chemical Industrial Complex in Russia discharged significant amounts of ^{32}P in the Yenisei river that were found to have accumulated in fish, with ^{32}P activities of 2.2 Bq/L in water and 2900 Bq/kg in fish found at 200 km from the

67 industrial site[4]. In the Columbia River contaminated by the cooling water from the Handford
68 plutonium reactors, the estimated level of ^{32}P in water between 1950 and 1971 varied from 0.1 to
69 7.7 Bq/L[5] and the activity of ^{32}P in fish was varied from 0.7 x 10^3 to 22 x 10^3 Bq/kg between 1962
70 and 1964[6]. Waterborne uptake of ^{32}P was found to be negligible as compared to uptake via the
71 dietary route in fish[7,8].

72 The 3-spined stickleback (*Gasterosteus aculeatus*) is a vertebrate model used in laboratory
73 settings to assess the mechanistic effects of pollutants and in field surveys using multi-biomarker
74 approaches[9-10]. The early stages of fish embryonic development are sensitive to ionising
75 radiation[11].

76 Numerous studies describe the effects of acute exposure to radiation on fish embryos. However,
77 much less is known about the effects of low doses of radiation in the environmental range of 0.1
78 to 10 mGy/day. Importantly, results from the literature on the effects of low doses on organism
79 health differ[12]. For instance, whilst some studies did not find evidence of radiation effects on
80 populations of aquatic invertebrates[13], fish[3] or mammals[14] at Chernobyl, others found adverse
81 effects of radiation on the abundance of invertebrates[15], birds[16] and mammals[17] at Chernobyl and
82 Fukushima. Thus, the dose at which significant damage to wildlife populations occurs remains an
83 open question[12].

84 Several studies have previously examined the effects of high doses of radiation (approximately
85 1000-fold above the environmental range) on the morphology of fish embryos. Exposure to acute
86 dose of radiation induced various morphological abnormalities in embryos of zebrafish (*Danio*
87 *rerio*)[18192021], mangrove killifish (*Kryptolebias marmoratus*)[22] and medaka (*Oryzias latipes*)[23].

88 Numerous authors studied the effects of exposure to high doses of radiation on the hatching
89 success of embryos but the results differed according to the type and nature of exposure. No

difference in the percentage of embryos that hatched was observed in medaka embryos exposed to 35.42 mGy/h of tritiated water[23] and in zebrafish embryos exposed to 0.01 and 0.05 Gy of γ radiation[21]. However, other studies found significant effects on the hatching success. The percentage of zebrafish embryos that hatched decreased significantly after exposure to a total dose range of 0.1 - 10 Gy[21,24], and to a dose rate range of 0.3 - 2 Gy/day of γ radiation[18]. The percentage of mangrove killifish embryos that hatched decreased significantly after exposure to a total dose range of 2.5 – 10 Gy[22]. The hatching time is significantly delayed as compared to controls in zebrafish embryos exposed to a dose range of 0.1 - 10 Gy[24] and a dose rate range of 0.3 - 2 Gy/day of γ radiation[18].

However, whilst many studies have examined the effects of high doses of radiation on hatching, fewer studies have examined hatching processes after exposure to lower doses, and no clear patterns of such dose effects has been observed. An acceleration of hatching was observed in zebrafish embryos exposed to a γ radiation dose of 10 and 1000 mGy/day, but no change was recorded after exposure to 1 and 100 mGy/day[18]. Hatching process appears to depend on the embryonic stage of exposure with some authors finding an acceleration of hatching in embryos exposed from 3 hpf (blastula stage) but not from 24 hpf (segmentation stage) to 1-1000 mGy/day of radiation[11].

Several studies investigated the effects of high doses of radiation on the cardiac physiology of fish embryos. Studies reported no effect[19] or a decreased heart rate [20] in zebrafish embryos. To our knowledge, no studies in the literature have yet described the heart physiology of embryos exposed to environmentally relevant doses of radiation.

Several recent studies explored the effects of short-term exposure to radiation (from a few seconds to 96 hrs) on the transcriptional response of embryos. Gene expression changes were

described in zebrafish embryos exposed to a total dose of 10 mGy to 100 mGy during 11 to 110 secs respectively (dose rate: 79.2 Gy/day), and the number of the differentially expressed genes was positively correlated to the dose[19]. Transcriptional response of genes involved in apoptosis[20] and DNA damage repair mechanisms[20,25] were changed in zebrafish embryos exposed to 0.5 - 4 Gy of ^{56}Fe ion irradiation (Si et al. 2017)[20] or 9.6 and 96 mGy/day of γ radiation[25].

The present study is one of the few to assess of the effects of very low doses of radiation on fish embryo development under laboratory conditions, combining both high throughput molecular and biometric analyses. The aim was to investigate whether exposure to environmental low doses of radiation induces developmental, physiological and transcriptional changes in stickleback embryos.

Methods

Fish maintenance

Adult sticklebacks were kept in artificial reproduction conditions in order to generate the embryos for *in vitro* fertilization. The artificial water composition and experimental conditions were selected according to the OECD guideline for the testing of chemicals ($CaCl_2$: 294, $MgSO_4$: 123, $NaHCO_3$: 65, KCl: 6 mg/L, pH = 7.5, T°C = 19°C, photoperiod: 16h light/8h dark)[26]. Four distinct couples were used to generate the embryos. The fertilization procedure was performed according to the protocol described in the OECD guideline for the testing of chemicals (test number 236). In total, 54 embryos were used for monitoring growth and physiology (27 controls and 27 exposed to the high dose), 108 for recording the hatching success (27 for each of the four exposure conditions), 104 for next generation sequencing (NGS) analyses (Table S3) and 36 to check ^{32}P uptake in the chorion, prolarvae and larvae (Table S2).

Three hours after fertilization, each embryo was placed in an individual glass tube and waterborne exposed to 3 mL of a radioactive solution of 0.1, 1 and 10 mGy/day (or 4, 40 and 400 μGy/h) using a solution of adenosine triphosphate labelled on the gamma phosphate group with ^{32}P (PerkinElmer). The final concentration of ATP in control, low and medium experimental tubes was adjusted to 1.1×10^{-5} μM by addition of stable ATP. This ATP concentration is negligible as compared to the concentration found in a typical cell of 5 mM[27]. The doses encompass the chronic low (L) (0.1 mGy/day) and medium (M) (1 mGy/day) doses encountered in the environment at Chernobyl 30 years after the accident, and the initial high dose (H) to fish after the accident (10 mGy/day). These doses span the Environmental Agency (EA) guidance level of 40 μGy/h (0.96 mGy/day) described in the radiological impacts on non- human species report of the EA in 2011[28]. Control embryos were kept in clean artificial water. 2.5 mL of the water was renewed every 3 days. The embryos were exposed for 10 days and euthanized according to schedule 1 of the Home Office Licence (ASPA, 1986)[29] using tricaine methanesulfonate (Sigma).

Dose calculation and monitoring

Dose at the centre of the hemisphere was calculated from data in Berger (1971)[30] (Figure S1). Activity of ^{32}P was measured using a HIDEX 300SL liquid scintillation counter and associated MikroWin 2000 software (Version 4.43).

Developmental and physiological measurements

The morphological parameters were measured using a Zeiss axiozoom microscope and the Zen Pro software. The physiological parameter was measured using an optical microscope. The growth of embryos was recorded at 4 dpf (days post-fertilization) by measuring the diameter (mm), area (mm^2) and eye distance (mm). The hatching rate was calculated as the proportion of fish that hatched to the total number of fish from the same condition for each observation time. The cardiac

159 physiology was assessed at 6 dpf by counting the heart beat rate (beats/min). The growth of larvae
160 was recorded at 10 dpf by measuring the length (mm) and head width (mm) of the larvae through
161 the glass tubes.
162
163 *Next generation sequencing (NGS)*
164 Differential expression analysis was conducted using NGS. For each of the 4 conditions (control,
165 low dose, medium dose and high dose), 3 biological replicates of pools of 4 to 10 embryos aged
166 of 10 days were used for NGS analyses (Table S3). Total RNAs were extracted using the High
167 Pure RNA Tissue kit (Roche Diagnostics Ltd, West Sussex, U.K.) according to the manufacturer's
168 instructions. RNA quality and integrity were evaluated using a bioanalyzer (Agilent, Santa Clara,
169 USA). RNA integrity numbers ranged from 7.4 to 9.5 and showed low RNA degradation rates.
170 NGS libraries for each pool were generated using the Illumina TruSeq mRNA library kit following
171 the manufacturer's instructions. Libraries were sequenced using the Illumina HiSeq 2500 analyser,
172 generating 125 base, paired-end sequences from libraries yielding an average of 33.4 ± 5.2 M
173 paired reads per sample (Table S4). Quality control of raw fastq reads was conducted using fastQC
174 (bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were trimmed using the Trim Galore
175 script to remove adapter sequences and low quality sequence tails
176 (bioinformatics.babraham.ac.uk/projects/trim_galore/). Trimmed reads were mapped against the
177 BROAD S1 *Gasterosteus aculeatus* 3-spined stickleback genome from Ensembl[30] using the STAR
178 universal RNA seq aligner[31] with parameters '--outSAMmultNmax 300'. Reads that mapped
179 uniquely to the genome in a proper pair with mapping quality score greater that 20 were used in
180 downstream analyses.
181 *Differential expression analyses*

Differential expression analyses were conducted using the DESeq2 package[33] in R. Gene models were taken from Ensembl version 82[31], and read counts over unique genes were quantified using the "summarizeOverlaps()" function in the GenomicAlignments package[34] using mode "Union". Raw read counts were normalised using the regularised log transformation in DEseq2 for visualisation (Figure S2). P values were adjusted for multiple testing by using the Benjamini and Hochberg correction[35]. To account for potential confounding effects of lineage due to a systematic difference in the pooling for replicate 3 compared to replicates 1 and 2 (Table S3), lineage was included as a covariate in the analysis. Significant differentially expressed genes were identified based on a fold-change of 2-fold or greater (up- or down-regulated) and an adjusted p-value less than 0.05. To avoid over-representing differential expression in low-abundance genes, significant genes were further filtered to remove those whose normalised expression was less than 1 for both the exposed and control groups. Gene ontology analysis was conducted using the clusterProfiler package[36].

Statistical analyses

Statistical analyses were performed using R version 3.1.2. After satisfying the assumptions of the normal distribution of the residuals, linear models were used to assess the potential differences. If the normality of the residuals wasn't respected a Kruskal-Wallis rank test was applied. A Fisher exact test was applied to assess any difference between hatching success at different times post-fertilization. When significant, post-hoc tests were performed and a Bonferroni correction of the α error was applied.

Results

Dose monitoring

The mean activity of ^{32}P solutions measured during the 10 days exposure experiment were 0.7 ± 0.2, 27 ± 3, 253 ± 24 and 2588 ± 199 Bq/mL (n = 12) (Table S1) and in accordance with the targeted activity of 2500, 250, 25 and 0 Bq/mL respectively, in each glass tube. After 3 days of exposure, the activities measured in the chorion were 1 ± 0 Bq for controls and low exposure conditions, and 3 ± 2 and 42 ± 11 Bq for the medium and high exposure conditions (mean ± SD, n = 3). The activities measured in dechorionated embryos were 1 ± 0 Bq for control, low and medium exposure conditions and 3 ± 2 Bq for the high exposure condition (mean ± SD, n = 3). After 9 days of exposure, the activities measured in the larvae were 1 ± 0 for control, low and medium exposure conditions and 2 ± 0 Bq for the high exposure condition (mean ± SD, n = 3) (Table S2).

Developmental and physiological parameters

Radiation exposure to 10 mGy/day did not significantly affect the growth of embryos. After 4 days, exposed embryos were equivalent in size. The diameter, surface and head width of exposed embryos were 1.46 ± 0.01 mm, 1.68 ± 0.02 mm^2 and 0.85 ± 0.03 mm respectively. These measures did not significantly differ from controls (diameter: 1.46 ± 0.01 mm, p = 0.65, area: 1.67 ± 0.02 mm^2, p = 0.64 and eye distance: 0.85 ± 0.03 mm, p = 0.17) (Table 1). After 6 days, the cardiac physiology of exposed embryos was not disturbed by exposure to radiation. The mean heart beats rate of exposed embryos were equal to 163.2 ± 12 beats/min and did not significantly differ from controls (150.0 ± 3.1 beats/min, p = 0.22) (Table 1). After 10 days, the development between exposed and control larvae remained similar. The length of exposed larvae was 5.98 ± 0.06 mm and their head width 0.82 ± 0.01 mm. These values did not significantly differ from controls (length: 5.99 ± 0.05 mm, p = 0.88 and head width: 0. 81 ± 0.01 mm, p = 0.48) (Table 1).

During the hatching process, embryos exposed to 1 (M) and 10 (H) mGy/day were slower to hatch at 170 hpf (hours post-fertilization), with 22% and 24% of the embryos respectively displaying a delay in hatching (p = 0.014 and p = 0.010 respectively) (Figure 1). At 174 hpf, the embryos exposed to the H condition reached 90% of hatching (HT_{90}) [169-176], 6 hours later than the controls that hatched at 168 hpf [164-169] (based on 95% confidence interval overlap) (Figure 2). There was no delay observed for the embryos exposed to L (HT_{90}: 171 hpf [168-173]) and M (HT_{90}: 172 hpf [166-175]) conditions as compared to the controls (Figure 2). Eventually, at 195 hpf, no significant difference in hatching success was observed between conditions (p = 0.058) (Figure 1) and the hatching percentage reached 100% [88-100] for the control embryos and 92%; 96% and 93% for the embryos exposed to 0.1 (L), 1 (M) and 10 (H) mGy/day conditions respectively (Figure 1).

Differential expression analysis

Analysis of the NGS data quality identified these data as showing excellent base calling qualities and post-filtering mapping rates of approximately 90% were seen throughout (Table S4). Following gene abundance identification, principal component analysis identified little difference between the dosage treatments in these data (Figure 3). A batch effect, resulting from the parentage of individuals pooled in the different replicates was found (Table S3). This batch effect was incorporated into the model for differential expression analysis.

Differential expression analysis was performed to identify genes whose expression was significantly deregulated following radiation exposure by comparing each of the three dosed treatments against a control treatment as described. No significant change in gene expression was observed, with only a single gene showing significant differential expression in the low dosage after filtering (Figure S2).

Data availability

The RNA sequencing data can be obtained from ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) with the accession number E-MTAB-7872.

Discussion

In the present study, embryo mortality was below 12% in each condition, in the range of what is considered as normal in studies using zebrafish embryos. In the literature, zebrafish embryos survival was reduced after exposures to higher doses such as 100 mGy[19] and after 2 and 4 Gy of exposure to ^{56}Fe[20].

During the hatching process, embryos exposed to 1 mGy/day (M) and 10 mGy/day (H) conditions displayed reduced hatching successes, by 22 and 24% respectively. No change was observed in embryos exposed to the lowest dose 0.1 mGy/day (L). At the end of the hatching process, no significant difference on the hatching success of embryos was noticed between controls and exposure conditions with percentages reaching 92% (L), 96% (M) and 93% (H).

Exposure to different dose and nature of radiation can accelerate hatching. For instance, zebrafish embryos exposed to 9.6 mGy/h for 65h[36], 0.3 to 2 Gy/day[18], 1-1000 mGy/day[11] and to an X-ray dose of 25 mGy[37] hatched earlier than control embryos. The embryonic stage at which radiation exposure occurs, appears to have consequences on the hatching sensitivity of embryos. For instance, mangrove killifish embryos displayed a higher sensitivity when exposed at an early stage to 2.5, 5, 7.5, and 10 Gy of γ radiation. In addition, the hatching success was significantly decreased in embryos that were exposed early (10.5 hpf) for all doses[22].

Other experiments showed that exposure to radiation induced a delay in hatching. Exposure to an X-ray dose above 25 mGy delayed hatching zebrafish embryos[38]. Waterborne exposure to 20

and 100 µg/L of ^{233}U induced a 12h delay in hatching time (HT_{50}: 59[54-66] and HT_{50}: 59[53-68] respectively), as compared to controls (HT_{50}: 47[45-48])[39]. In our study, exposure to waterborne ^{32}P induced a 6h delay between embryos exposed to the highest dose, 10 mGy/day (H), (HT_{90}: 174 hpf [169-176]) as compared to controls (HT_{90}: 168 hpf [164-169]). A study perfomed on zebrafish embryos did not observe any modification of the hatching success of embryos exposed to a radiation dose range of 1, 2, 5 and 10 Gy[40].

During hatching, biochemical and behavioural process are synchronised to destroy the chorion[41]. Proteolytic enzymes and embryos movement contribute to the chorion disruption to allow hatching. Hatching may be delayed because of potential changes induced by radiation to those enzymes, as evidenced by studies on the effects of copper on rainbow trout eggs[41]. The delay could also reflect a protective response to stress where the chorion would protect the embryos from external hazard. A similar delay has been reported in zebrafish embryos exposed to metals[42,43]. The present data suggest that a delay in hatching is a good indicator of exposure to environmental low dose of radiation in laboratory settings, which is in agreement with the study by Bourrachot et al (2008)[39] that uses waterborne ^{233}U. A developmental delay of maturing fish eggs has also been observed in organism exposed in their natural environment. A delay of oocyte growth has been evidenced in perch exposed to a total dose rate of 10-16 µGy/h (0.2-0.4 mGy/day) in exposed lakes at Chernobyl, 30 years after the accident, and was correlated to the radiation dose[3]. The precise mechanism by which radiation induces this delay is unknown. At the molecular level, a recent study found that transcriptional response of genes involved in the circadian clock was modulated in zebrafish larvae (at 96 hpf) exposed to 0.4 and 4 mGy/h of tritiated water[25].

In the present study, radiation exposure to 10 mGy/day did not significantly affect the growth of embryos (no difference of diameter, area and eye distance at 4 dpf) and larvae (no difference of

296 head width and length at 10 dpf) respectively. Similarly, no deformity (short tail, spinal curve,
297 absence of pigment, failed hatching) and no length difference as compared to controls was
298 observed in embryos exposed to 9.6 mGy/h[37]. A recent environmental study found that the length
299 and Fulton condition index of perch and roach were similar between lakes, in addition, no
300 malformation of gonads and oocytes was recorded revealing that fish were in good health in
301 general[3]. Other studies found a tail detachment in zebrafish exposed to 20 µg/L of ^{233}U but not in
302 embryos exposed to 100 µg/L of ^{233}U and a decrease of the body length for both exposures[39]. A
303 reduction of body length was also found in zebrafish embryos exposed to high dose of radiation
304 (1 - 10 Gy)[40,24]. Exposure to a γ radiation dose of 0.3 to 2 Gy/day induced morphological
305 abnormalities (tail atrophia and trunk axis malformations) in zebrafish embryos[18]. Using an acute
306 dose of radiation, deformities (including spinal curvature, pericardial cyst enlargement and
307 thoracic cavity variation) were noticed from 0.1 Gy, and hatching was reduced from 0.05 Gy[19].
308 Malformations such as tail deformity, pericardial edema and spinal curve were found to increase
309 in zebrafish embryos exposed to 2 and 4 Gy of ^{56}Fe ion irradiation[20] and 0.01 to 1 Gy of γ
310 radiation[21]. Pericardial and yolk sac edema, curved notochord and thin caudal fin were observed
311 in the hermaphroditic fish embryos exposed to a total dose range of 2.5 - 10 Gy of γ radiation[22].
312 Vertebral malformations were reported in medaka embryos exposed to 35.42 mGy/h of tritiated
313 water[23]. Only a few studies have looked at the head development. Freeman et al. (2014)[40] found
314 that exposure to high dose of radiation (10 Gy) reduced eye diameter and head length. However,
315 this dose is higher than the environmental dose range used in the present study.
316 The physiology of 6 dpf embryos exposed to 10 mGy/day was not changed as compared to
317 controls based on the heart beats count. This is in agreement with a few previous studies that did
318 not observed any change in zebrafish embryos exposed to a total dose of 10 to 100 mGy for 10.9

s to 109 s respectively (dose rate of 79.2 Gy/day)[19] or after exposure to 2 Gy of ^{56}Fe ion irradiation (dose rate: 0.5 Gy/min)[20]. The present work represents one of the rare studies that have assessed this physiological criterion at environmental low doses.

Interestingly, no significant transcriptional changes were observed in 10 dpf larvae after exposure to all dose levels. This may be a result of genes involved in protective mechanisms already being activated before the larval stage to compensate for the negative effects of radiation exposure, but returning to normal levels in later stages of development. In a recent transcriptomic study assessing the effects of tritiated water on zebrafish embryos, it was suggested that the onset of an early protective mechanism against oxidative stress may not be observed at the larval stage of development (96 hpf)[25]. Indeed, antioxidant defence mechanisms that are activated in embryos may lead to a decrease in lipid peroxidation[37] and a reduction in DNA damage[44].

Another hypothesis is that the environmental doses used in the present study may be too low for eliciting a differential gene expression change. The basal gene transcriptional levels may be sufficient for the larvae to account for the effects of radiation. Results from the literature indicate a change in gene expression for higher exposure. Kumar et al (2017)[24] found that exposure to 5 Gy of radiation induced transcriptional changes of *sox* genes involved in development. The expression of genes seemed dependent on the embryonic stage of development and the dose level. Transcriptional changes of genes involved in antioxidant defence were found in early stage zebrafish embryos exposed to low doses, but not to high doses. These gene transcriptional levels were unchanged at a later stage for both exposure conditions[25]. The environmental doses used in the present study and currently existing at Chernobyl may be too low to induce a significant oxidative stress[45] and subsequent DNA damage. No genotoxic effect was evidenced as measured

by micronuclei in erythrocytes of perch, roach[3] and catfish[46] exposed to environmental radiation at Chernobyl.

These data suggest that low levels of radiation exposure have a negligible effect on gene expression profiles and embryo growth and physiology but result in subtle delays to hatching times that does not affect the final numbers of fish that hatched. The current levels of environmental radiation at NPP sites are therefore unlikely to negatively impact embryonic development of future offspring. These results support the findings from a previous large-scale environmental study that found a delay in the maturation of perch eggs, and that fish were otherwise in good general health[3]. Moreover, these results corroborate other environmental studies led at Chernobyl on aquatic macro-invertebrate development and physiology[45,46]. Finally, this laboratory study is important as it provides environmentally relevant data to refine the current thresholds for which an effect is observed.

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Figure and Table Legends.

Table 1. Morphological and physiological parameters recorded on 4 and 6 dpf embryos and 10 dpf larvae (mean ± SEM, *n* =27).

Figure 1. Hatching success of embryos exposed to control, Low (0.1 mGy/day), Medium (1 mGy/day) and High (10 mGy/day) conditions, recorded at 166, 168, 172 and 195 dpf (%, IC95, *n* = 27).

Figure 2. Time necessary to reach 90% of hatching for embryos exposed to control, Low (0.1 mGy/day), Medium (1 mGy/day) and High (10 mGy/day) conditions (*n* = 27, IC95).

Figure 3. Principal component analysis showing the distribution of the 12 samples according to their gene expression profile over the top 500 genes based on their variance.

Table 1.

Morphological and physiological parameters	Exposure condition	
	Control	10 mGy/day
Embryos 4 dpf		
Diameter (mm)	1.46 ± 0.01	1.46 ± 0.01
Area (mm^2)	1.67 ± 0.02	1.68 ± 0.02
Eye distance (mm)	0.91 ± 0.02	0.85 ± 0.03
Embryos 6 dpf		
Heart beats (beats/min)	150.0 ± 3.1	163.2 ± 12
Larvae 10 dpf		
Head width (mm)	0.81 ± 0.01	0.82 ± 0.01
Length (mm)	5.99 ± 0.05	5.98 ± 0.06

Figure 1.

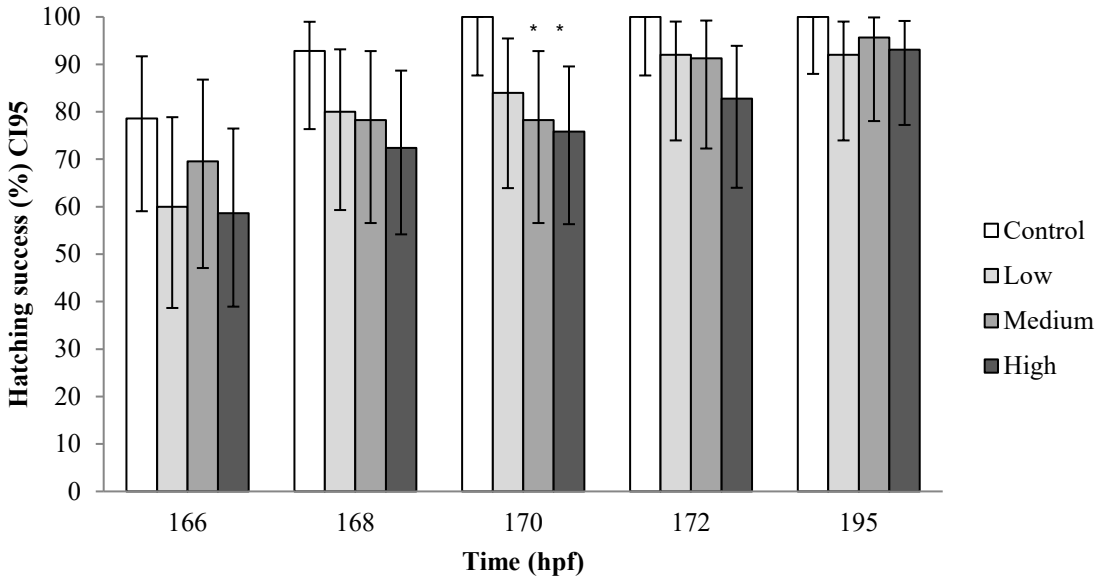

Hatching success (%) CI95
100
90
80
70
60
50
40
30
20
10
0
166
168
170
172
195
Time (hpf)
* *
Control
Low
Medium
High

Figure 2.

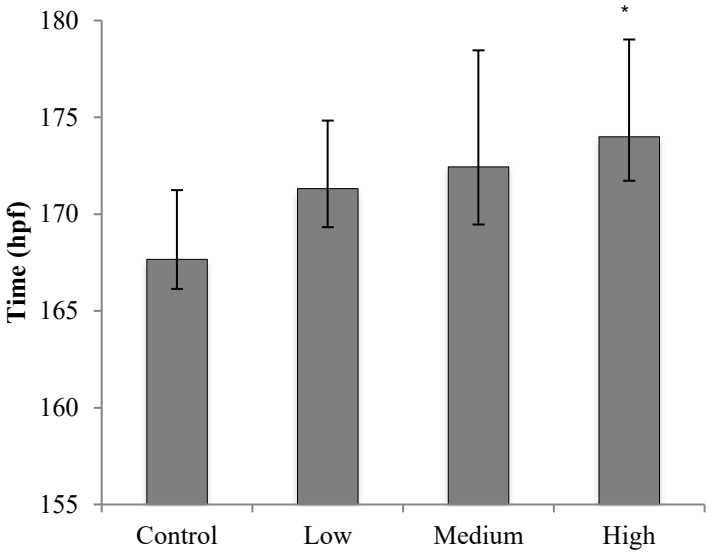
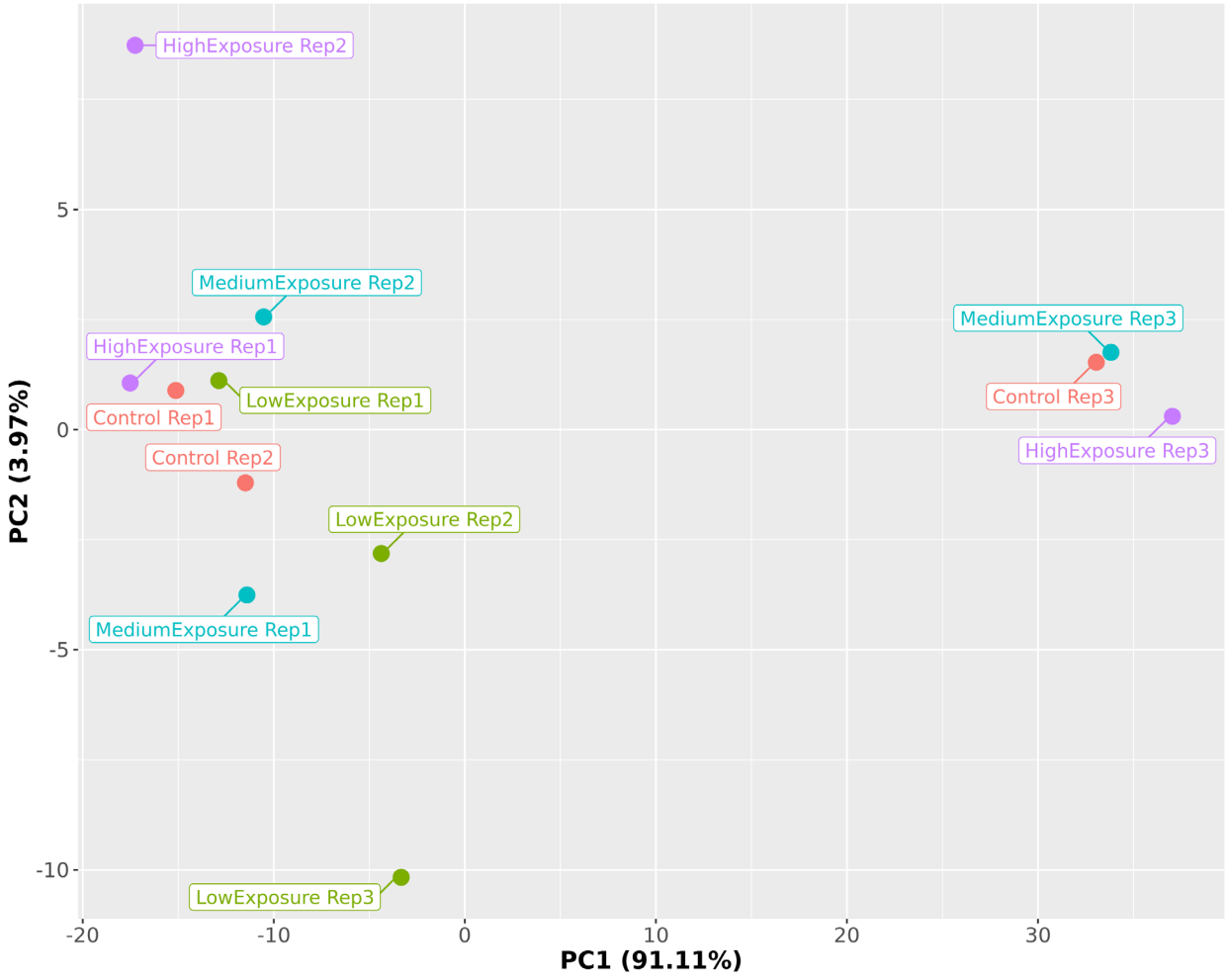


Figure 3.


HighExposure Rep2
MediumExposure Rep2
HighExposure Rep1
LowExposure Rep1
Control Rep1
Control Rep2
LowExposure Rep2
MediumExposure Rep1
LowExposure Rep3
MediumExposure Rep3
Control Rep3
HighExposure Rep3
PC2 (3.97%)
PC1 (91.11%)
5
0
-5
-10
-20
-10
0
10
20
30