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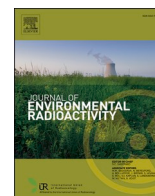
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Common and inter-specific toxic effects in three wild fish species after chronic gamma irradiation of early stages

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ABSTRACT

The objective of this study was to investigate the effects of gamma irradiation on the aquatic environment. We used three wild fish species to compare phenotypic responses with a fish model such as *Danio rerio*. We focused on embryonic development, a sensitive life stage to stressors like ionizing radiation, to evaluate the effects of exposure to 0.5 and 5 mGy h⁻¹ on Arctic char, trout and stickleback embryos from fertilization to free-swimming larvae. Irradiation did not cause mortality but induced an acceleration of hatching in the three species. These new data on wild species, obtained under comparable irradiation conditions, did not go against the threshold values for the protection of freshwater aquatic ecosystems. Moreover, irradiation caused inter-specific sublethal effects, such as an increase in non-eyed egg proportion in Arctic char, an increase in the incubation period in trout and an acceleration of larval mortality in stickleback. The consequences of these early effects on the adult stage remain to be studied.

1. Introduction

Ecological risk assessment is necessary for the control and prevention of harmful effects of pollutants on humans and non-human species. Firstly, mortality, morbidity (including visible abnormalities) and reproduction of individuals are currently the main endpoints used to assess effects for ecological risk assessment because they affect the population growth rate or structure (EC-TG N°27, 2011; ICRP 124, 2014). Secondly, the fish embryo test (FET, OECD guideline 236) was validated given its excellent correlation with the acute fish toxicity test based on juvenile fish (OECD TG 203) and adopted as a standard test method for fish toxicity test (von Hellfeld et al., 2020; Wang et al., 2021). These tests are commonly used to assess the toxicity of chemical pollutants. However, very few were used to study the effects of ionizing radiation (IR) on fish, known to induce oxidative stress and DNA damages. Nevertheless, ICRP 108 provides a few heterogeneous radiation-induced mortality data (adult, embryo) on salmonids, roach, guppy, medaka, goldfish and mosquito fish. These few data made it possible to propose Derived Consideration Reference Levels (DCRL) for the protection of the environment, defined as a band of dose rate within which there is likely to be some chance of deleterious effects of ionizing radiation occurring to individuals of that type of Reference Animals and Plants (RAP) (Coppelstone et al., 2020). The DCRL varies from 40 to 400

μGy h⁻¹ for pelagic freshwater fish. However, it is known that IR-induced phenotypical effects vary depending on species, and that they are dependent on the exposed embryo-larval stage (morula, gastrula, etc.), the dose rates and the total dose absorbed by the organisms, which make inter-specific comparison difficult (ICRP 108, 2008). Given the available data, it seems necessary to increase knowledge to provide a robust radiotoxicity threshold.

Additional data have recently been obtained for the fish model, *Danio rerio*. Zebrafish is an ideal model to address the effects of stress on the early embryo, given that their eggs are fertilized externally and can be easily observed throughout early development. Their rapid development into free-swimming larvae within the first three days of life also makes them suitable for high-throughput experiments (Geffroy and Simon, 2013; Lawrence, 2007). Gamma irradiation resulted in significant effects on zebrafish embryo-larval endpoints from dose rates as low as 42–50 μGy h⁻¹ to 42–50 mGy h⁻¹, mainly influencing hatching, resistance to starvation, biomass, yolk bag diameter in connection with DNA damages and oxidative stress (Gagnaire et al., 2015; Hurem et al., 2017; Jarvis and Knowles, 2003; Pereira et al., 2011; Simon et al., 2011a). More recently, gamma irradiation (>0.54 mGy h⁻¹) during early gastrula caused gene expression changes linked to developmental impairments in zebrafish embryos (Hurem et al., 2017). Motility was decreased and expression of genes involved in mitochondrial activity

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and in the neuromuscular developmental pathway were modified at dose rates greater or equal to 0.5 mGy h⁻¹ (Houdigui et al., 2020; Murat El Houdigui et al., 2019). However, ionizing radiation does not affect early-stage mortality (except after parental F0 irradiation (5–50 mGy h⁻¹) (Guirandy et al., 2019, 2022)). Given the short incubation period of zebrafish embryos, the cumulative dose during irradiation remains low, between 3.6 mGy and 3.6 Gy. Based on this evidence, we hypothesize that the incubation period of this tropical species presenting a short development time is not sufficient to reach high cumulative doses that could lead to more drastic effects.

The present study aimed to evaluate the organism-level endpoints (mortality, abnormality, developmental, hatching) to gamma radiation by using three different wild cold fish species (reproduction <12 °C): Arctic char, rainbow trout and stickleback. This objective was to compare phenotypical endpoints after higher cumulative doses than the one obtained for zebrafish. The early stages of the three selected species were exposed chronically to irradiation soon after fertilization through development until the resorption of yolk bag. In addition, Arctic char and trout embryos were exposed at late developmental stages to determine critical irradiation windows responsible for the potential appearance of effects. This work proposes (i) to evaluate the link between cumulative dose received by the early stages of selected fish species and toxicity of IR and (ii) to compare inter-specific responses of these three wild fish species and of zebrafish.

2. Materials and methods

2.1. production and experimental design

Eggs of the Arctic char, *Salvelinus alpinus* (twenty-one males (1.1 ± 1.2 kg body weight and 40.1 ± 1.1 cm total length) and fifteen females (3.7 ± 0.9 kg body weight and 42.4 ± 1.7 cm total length)), were obtained from brood stock by INRAE-CARRETEL agreement number: E74 300–4. Eggs of rainbow trout, *Oncorhynchus mykiss*, and three-spined stickleback, *Gasterosteus aculeatus*, were obtained from a commercial supplier and INERIS, respectively. All eggs came from the artificial reproduction of wild fish. Continuous egg irradiation began 6 h after fertilization for the two salmonid species (*S. alpinus*, *O. mykiss*) and two days after fertilization for *G. aculeatus*, related to transport time (Table 1). Four groups of different breeder three-spined sticklebacks (*in vitro* fecundation, IVF) were used to produce four batches of eggs. To reduce the impact of the genetic pool, each batch of eggs was then separated into two groups, the first being introduced under control condition, the second under irradiation condition. The three-spined stickleback has a unique reproductive mode including male nest-building with a secreted glue-like protein (spiggin) (Jakobsson et al., 1999). Therefore, the eggs were manually individually isolated. Eggs of

three species were individually placed in 24-well culture plates containing 3 ml of aerated Volvic natural mineral water under constant temperature (5 °C for both salmonids, 19 °C for three-spined stickleback) to meet the optimal temperature of growth (Table 1). Under these experimental conditions, it seemed difficult to feed all the larvae uniformly. Therefore it was decided not to feed the hatched larvae, as the experiment was stopped when the resorption of yolk-bag was achieved. Nominal dose rates were 0.5 and 5 mGy h⁻¹ in connection with experiments previously carried out on the model fish *D. rerio* (Simon et al., 2011b). In addition, we irradiated a second batch of older embryos, 48 d-old trout embryos (n = 72) and 108 d-old Arctic char embryos (n = 24) under two irradiation conditions (0.5 and 5 mGy h⁻¹) to monitor phenotypic responses. Control conditions were kept in a separate room (60–80 nGy h⁻¹).

Gamma irradiation was performed on the Micado'Lab experimental platform (IRSN) from a ¹³⁷Cs source (444 GBq, 662 keV) (Guirandy et al., 2022). Dose rate was simulated using MCNP5 software and measured using thermoluminescent dosimeters (Chiyoada Technologies), and the values represented between 80 and 120% of the nominal values.

2.2. Endpoints

Measured endpoints included embryo and larval mortalities, hatching success (<https://www.aatbio.com/tools/ec50-calculator>), morphological abnormalities, development, in particular eye formation (eyed egg stage), size of the yolk reserve and mass/length. These main endpoints were scored every two days for each species for the three exposure conditions. For Arctic char, fresh mass was measured for eggs at time 14 d, 21 d, 56 d, 77 d and for larvae at 115 d. The total length and length and width of the yolk sac of eggs/larvae (n = 10/condition) were measured at time 94 d (except for the 0.5 mGy h⁻¹) and 102 d. For trout, fresh egg mass was measured at time 50 d and 68 d (n = 20). Individual stickleback egg mass was low, requiring special analytical tools that were not available in the laboratory. The total length and length and surface of the yolk sac of eggs/larvae were measured at time at 96 d (n = 43–45) and 112 d (n = 41–49). Images analysis to measure body length were performed with DanioScope v1.1.110 (Noldus Information Technology, Netherlands).

2.3. Statistical analyses

The number of alive and dead larvae was recorded and the respective percentages per species calculated. The differences in the mortality ratios between treatments were analysed for the three species with a contingency table and tested with a Chi² proportion test. The survival rate was calculated for each treatment using survival analysis with Cox

Table 1

Species, irradiation exposure (dose rate, mGy h⁻¹, cumulative dose, Gy), effective per condition, temperature and exposure duration (day). d = day, hpf = hour post fertilization, dpf = day post fertilization.

Species	Control		0.5 mGy h ⁻¹		5 mGy h ⁻¹		Duration (d)	T °C	Age at start of exposure	
	Effective	Cumulative dose (Gy)	Effective	Cumulative dose (Gy)	Effective	Cumulative dose (Gy)			hpf	dpf
Arctic char <i>S. alpinus</i>	96		96		96		140	5	6	
	24	0.2	24	1.7	24	16.8		5		108
Trout <i>O. mykiss</i>	192		192		192		112	5	6	
	72	0.2	72	1.3	72	13.4		5		48
Stickleback <i>G. aculeatus</i>	165	0.04			169	3.0	25	19		2

Proportional Hazards Models for treatment. For sticklebacks, the IVF variable was also considered in the analyses but had no effect on the differences observed between the different treatments. The analyses were performed using R software (R Core Team, 2013) with the following packages: “forcats”, “survminer”, “lme4”, “survival”. The threshold chosen for significant results was 0.05. Irradiation effects on mass and length were assessed using a non-parametric test (Kruskal-Wallis test).

3. Results

3.1. Similar/common effects

3.1.1. No significant effect of irradiation on mortality

For trout and stickleback, the rate of dead eggs was low, less than 8% at the first hatching and increased to a maximum of 14% (Fig. 1A&B). For Arctic char, mortality rate was significantly higher. First, for the controls, mortality started abruptly (around 10%) on the 77th day, several days before the first hatch (91 d), whereas it was low for the other two conditions (<2% at 77 d). Second, the state of progress of embryonic development revealed two populations of eggs (eyed and non-eyed egg populations). Unfortunately, the mortality rate of these two egg populations was not monitored, only the overall egg mortality rate was measured. Exposure to gamma irradiation did not lead to an increase in mortality for any tested species. Egg mortality at 0.5 mGy h⁻¹ seemed lower than control and high irradiation exposures.

Moreover, exposure to gamma irradiation did not result in an increase larval mortality, for any tested species (Fig. 1C). Compared to egg mortality, larval mortality was lower than egg mortality except for the Arctic char at 0.5 mGy h⁻¹.

3.1.2. Acceleration in hatching time

Key hatch times have been expressed as the times necessary for the first hatch, 10%, 50% of the population hatched and the last hatch (HT100) (Fig. 2). For Arctic char and stickleback, irradiated eggs hatched significantly earlier than control eggs. It was the same for trout, except for the time necessary to reach 100% of hatched eggs. No dose effect appeared to be significant. The cumulative doses (dose rate x exposure duration) at the first and last hatching were comprised between 10 and 12 Gy for Arctic char, 8.4 and 13.4 Gy for trout and 0.72 and 1.05 Gy for stickleback.

Tests to question the existence of a critical irradiation window were carried out for the two species of Salmonids (Fig. 3). The effects of late irradiation were addressed. Results were mixed: for Arctic char, no effect on the hatching duration was highlighted, whereas for trout, the acceleration of the hatching process for the two dose rates was once again observed.

3.1.3. Transitory effect on larval mass and length

Significant effect on larval mass ($p = 0.0004$) was observed only at 115 d, for 0.5 mGy h⁻¹ condition for Arctic char (Fig. 4).

For the Arctic char, a significant difference of total length was

observed at time 102 d for the 0.5 mGy h⁻¹ condition and from time 94 d for the 5 mGy h⁻¹ condition (Fig. 5A). For trout, significant differences on larval length were observed at time 96 d for the high dose rate and at time 112 d for the low dose rate (Fig. 5B).

In parallel with these effects common to the three species, specific effects to each species were observed.

3.2. Inter specific endpoints

All of the effects described in this section have been researched for the three species of fish.

3.2.1. Arctic char, *S. alpinus*

The main effect concerned the proportion between eyed and non-eyed egg populations (Fig. 6). At time 77 d, eight days before the first hatching, 35% of the eggs of the 5 mGy h⁻¹ condition were not eyed, whereas 11.5% and 19.8% were in the control and 0.5 mGy h⁻¹ conditions, respectively. The mortality kinetics of non-eyed eggs were different from those of eyed eggs; firstly, non-eyed eggs lived significantly longer than eyed eggs and secondly, the non-eyed eggs hatched into live larvae to 0%, 22% and 17% for control, 0.5 and 5 mGy h⁻¹ conditions, respectively. Such effect was not observed in the other two species.

3.2.2. Trout, *O. mykiss*

The duration of hatching (defined as the time between the first hatching and the last hatching) was greatly lengthened in trout. The two irradiated populations took 37 d and 42 d to hatch against only 21 d for the control condition. For the two other fish species, even if the progress of hatching was accelerated, the total duration of hatching was not impacted.

Abnormal larvae were observed only in trout. They present either a reduced size, or malformations (cardiac or yolk sac edema) (Fig. 7A) or hatched larvae without eliminating the chorion; the larva could move but the head remained stuck in the chorion (Fig. 7B). This last category represented at most 72% and 93% of abnormal larvae for 0.5 mGy h⁻¹ and 5 mGy h⁻¹ conditions respectively. No such larvae were observed in the control condition. On day 100, 26 and 28 larvae were trapped in the chorion for 0.5 mGy h⁻¹ and 5 mGy h⁻¹ conditions, respectively. At day 122 d, 70% of these larvae had hatched (Fig. 7C). No significant dose effect was found.

3.2.3. Stickleback, *G. aculeatus*

Remember here that the eggs from the same IVF were separated into 2 groups, the first being irradiated, and the second being in control condition. The period during which the unfed larvae were alive was longer in the control larvae than in the irradiated larvae (Fig. 8). The duration between maximum hatching rate and the onset of mortality was 14 days for the four controls and 9–10 days for the irradiated condition.

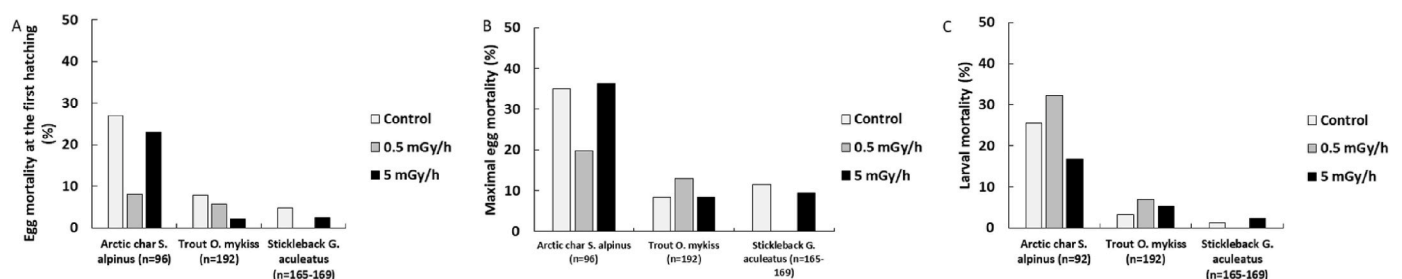


Fig. 1. Egg mortality at the first hatching (A), maximal egg mortality (B) and larval mortality (C) for 3 wild fish species exposed to 0, 0.5 and 5 mGy h⁻¹ gamma radiation. No significant differences between treatments could be identified based on a Chi² proportion test (* $p < 0.05$).

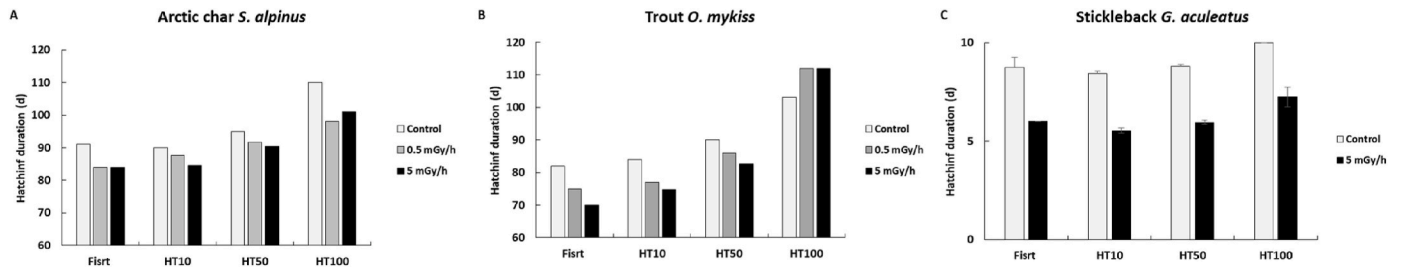


Fig. 2. Time to reach the first hatch and to reach 10, 50 and 100% hatching for 3 wild fish species exposed to 0, 0.5 and 5 mGy h⁻¹ gamma radiation, <https://www.aatbio.com/tools/ec50-calculator>.

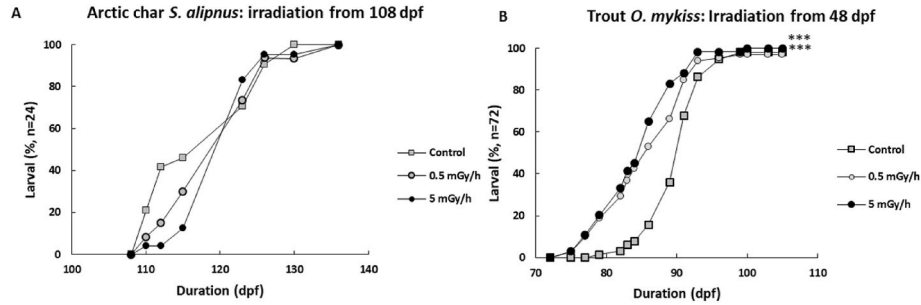


Fig. 3. Kinetic of hatches (%) over time from 108 dpf for Arctic char (A) and for 48 dpf for trout (B) exposed to 0, 0.5 and 5 mGy h⁻¹ gamma radiation. Statistical analyses were performed using a Cox proportional hazards Model (*p < 0.05, **p < 0.01, ***p < 0.001).

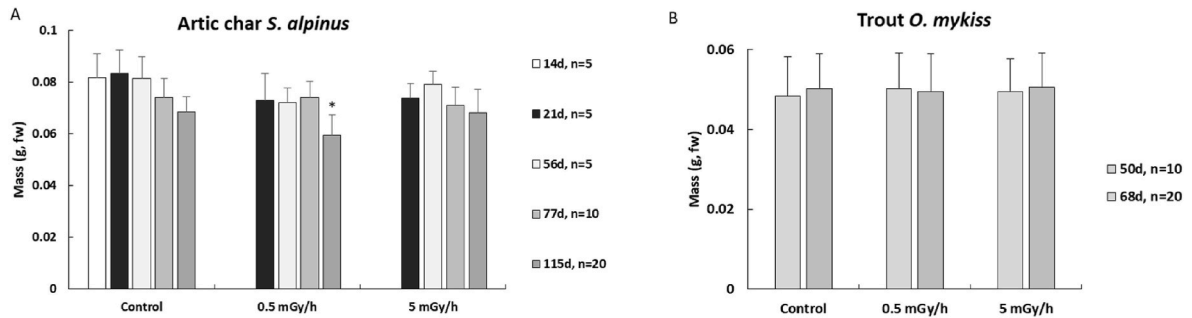


Fig. 4. Mass (g, fw) of individual egg and larval over time for Arctic char *S. alpinus* (A) and trout *O. mykiss* (B) exposed to 0, 0.5 and 5 mGy h⁻¹ gamma radiation. n = effective per condition. Statistical analyses were performed using a Kruskal-Wallis test (*p < 0.05).

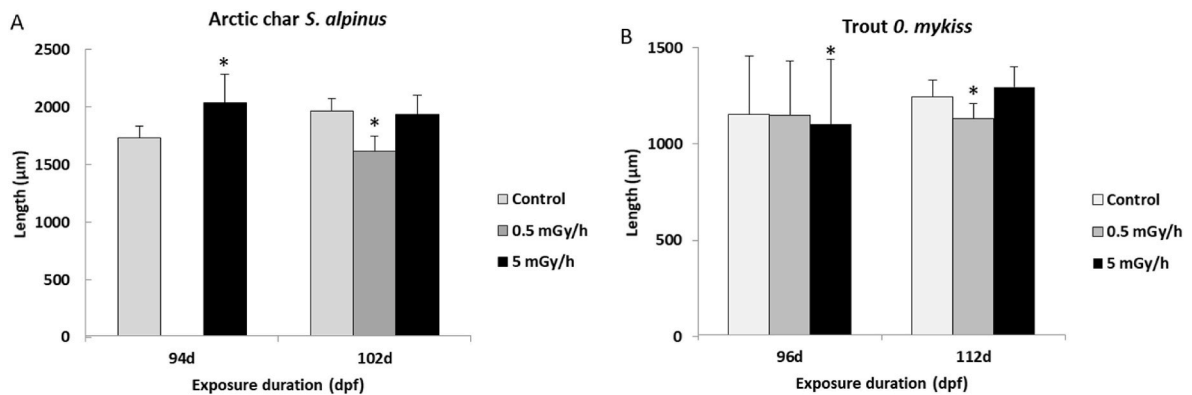


Fig. 5. Length (μm, n = 10) of larval for Arctic char *S. alpinus* (A) and trout *O. mykiss* (B) exposed to 0, 0.5 and 5 mGy h⁻¹ gamma radiation. Statistical analyses were performed using a Kruskal-Wallis test (*p < 0.05).

4. Discussion

Sticklebacks (Cant et al., 2022; Lebeau-Roche et al., 2023; Marchand

et al., 2021) and trouts are the subject of many ecotoxicological studies, unlike Arctic char (Réalis-Doyelle et al., 2023). However, few studies concern the comparison of the phenotypic responses of wild species

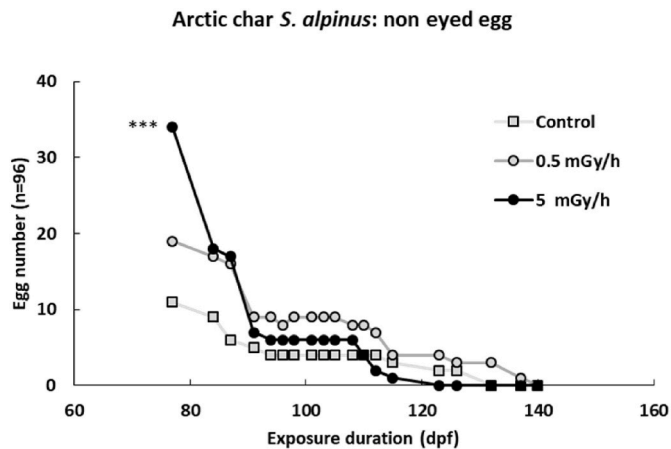


Fig. 6. Number over time of the non-eyed eggs for Arctic char *S. alpinus* (A) exposed to 0, 0.5 and 5 mGy h⁻¹ gamma radiation. Statistical analyses were performed using a Cox proportional-hazards Model (*p < 0.05, **p < 0.01, ***p < 0.001).

under comparable laboratory irradiation conditions. Here, an absence of radiation-induced mortality and an acceleration of hatching were commonly observed in the three species of fish.

4.1. Mortality

Ionizing radiation is known to induce DNA lesions directly or indirectly by formation of hydroxyl free radicals. The type of induced DNA lesions are double strand breaks (DSBs) and single strand breaks (SSBs) (Guo et al., 2013; Honjo and Ichinohe, 2020; Simon et al., 2011b). At high dose rate (5 mGy h⁻¹), trout and Arctic char eggs received 8.4 and 10 Gy, respectively, at the time of first hatching. Despite this high cumulative dose, no significant egg or larvae mortality was found. Moreover, mortality rate was not affected by older embryonic stage exposure. For stickleback, which has a faster development time (0.7 Gy at the first hatching), likewise both salmonids, no significant mortality was observed. The same result was observed with zebrafish, which presents a faster embryo-development compared to stickleback (Simon et al., 2011b). The present results are therefore in agreement with literature data. Mortality in embryo from RAP “Reference Trout” is induced for dose rate higher than 42 mGy h⁻¹ (ICPR 108, 2008). Available data show great variability in LD₅₀ values (ICPR 108, 2008) in connection with the irradiation conditions, in particular the choice of the age of the irradiated embryo-larval stage. In Chinook salmon, LD₅₀ values varied from 3.4 to 10 Gy when irradiation (x-rays) was performed at the single cell or at eyed embryo stages (ICPR 108, 2008). In general, few studies have been carried out on very early stages of wild species, certainly in connection with the experimental difficulties in transporting and maintaining a high survival rate in controls. Indeed, ionic composition

of freshwater exposure can have strong effects on salmonid emergence, with large difference on laboratory survival rate (Sternecker and Geist, 2010). Egg survival of hatchery Arctic char is unpredictable and often poor mainly in connection with female age (Jeuthe et al., 2016) or underlying genetic components (Palaiokostas et al., 2021). Laboratory studies of the early stages of Arctic char are rare (Devaux et al., 2011; Lubin et al., 2024; Réalis-Doyelle et al., 2023). However, the recent study of Réalis-Doyelle et al. (2023) showed laboratory mortality rates comparable to those measured in the present study. Conversely, the choice of model species of fish, such as the zebrafish or medaka, with low mortality rates (<20%) makes possible the early study of irradiation (from 1 hpf or 3 hpf) (Arcanjo et al., 2018; Geffroy and Simon, 2013; Guirandy et al., 2019, 2022; Honjo and Ichinohe, 2020; Murat El Houdigui et al., 2019; Praveen Kumar et al., 2017). Although embryos are generally very sensitive to radiation, sensitivity varies across different stages of embryonic development. This is why it seems necessary to study the effects of irradiation at the earliest stages to gain ecological robustness (Honjo and Ichinohe, 2020). Stickleback and trout mortality rates in the control condition were low; on the other hand, mortality rate of Arctic char was high (20–30%), definitely related to parental effects on offspring development, in particular early mortality as previously demonstrated (Eilertsen et al., 2009; Janhunen et al., 2010). The use of animals for development of a wild embryo-larval bioassay should be reserved for species with a low mortality rate in laboratory conditions.

This work confirmed the absence of radiation-induced mortality for the wild fish egg stage (5 mGy h⁻¹, 3–13.4–16.8 Gy) as observed in *Danio rerio* at the same dose rate (5 mGy h⁻¹) but at a much lower cumulative dose (0.36 Gy) (Gagnaire et al., 2015; Guirandy et al., 2019; Simon et al., 2011b). As the exposure duration of different biological stages and the lifespan of organisms vary considerably, the dose rates associated with the biological cumulative doses could be used to express the radio-toxicity encountered in natural environment. Nevertheless, we can issue the hypothesis of sublethal attacks on the second generation, as previous studies have demonstrated on zebrafish. Indeed, the sensitivity of zebrafish egg stage appeared when the irradiated eggs came from parents themselves irradiated (Guirandy et al., 2019, 2022). The continuation of transgenerational effects will be difficult to set up for wild species with a long development to reach sexual maturity.

4.2. Hatching

Few data exist regarding the fish egg response to assess the toxicity of various pollutants. Hatching appears to be a sensitive phenotypic marker of exposure (Bourrachot et al., 2008; Frayse et al., 2006; Stasiūnaitė, 1999; Weeks Santos et al., 2021; Zhang et al., 2018). Here, the acceleration due to irradiation compared to control at the first hatching was around 8%, 15% and 31% for Arctic char, trout and stickleback at the highest dose, respectively. The hatching of larvae is an important point in the life cycle of fish, and a combination of biochemical and physical mechanisms regulates the process (Frayse et al., 2006), which happens physiologically between 48 hpf and 72 hpf

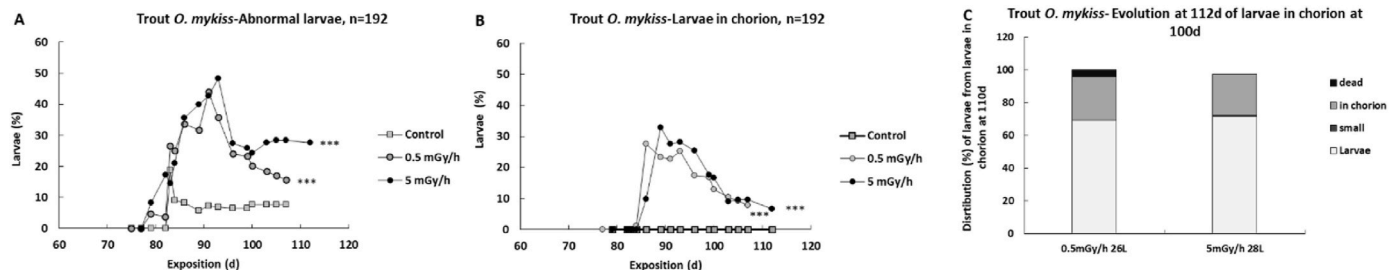


Fig. 7. Abnormal larvae (A) and hatched larvae remaining in the chorion (B) over time for the 3 conditions and become larvae in the chorion between 100 and 12 d (C) for trout *O. mykiss* exposed to 0, 0.5 and 5 mGy h⁻¹ gamma radiation. Statistical analyses were performed using a Cox proportional-hazards Model (*p < 0.05, **p < 0.01, ***p < 0.001).

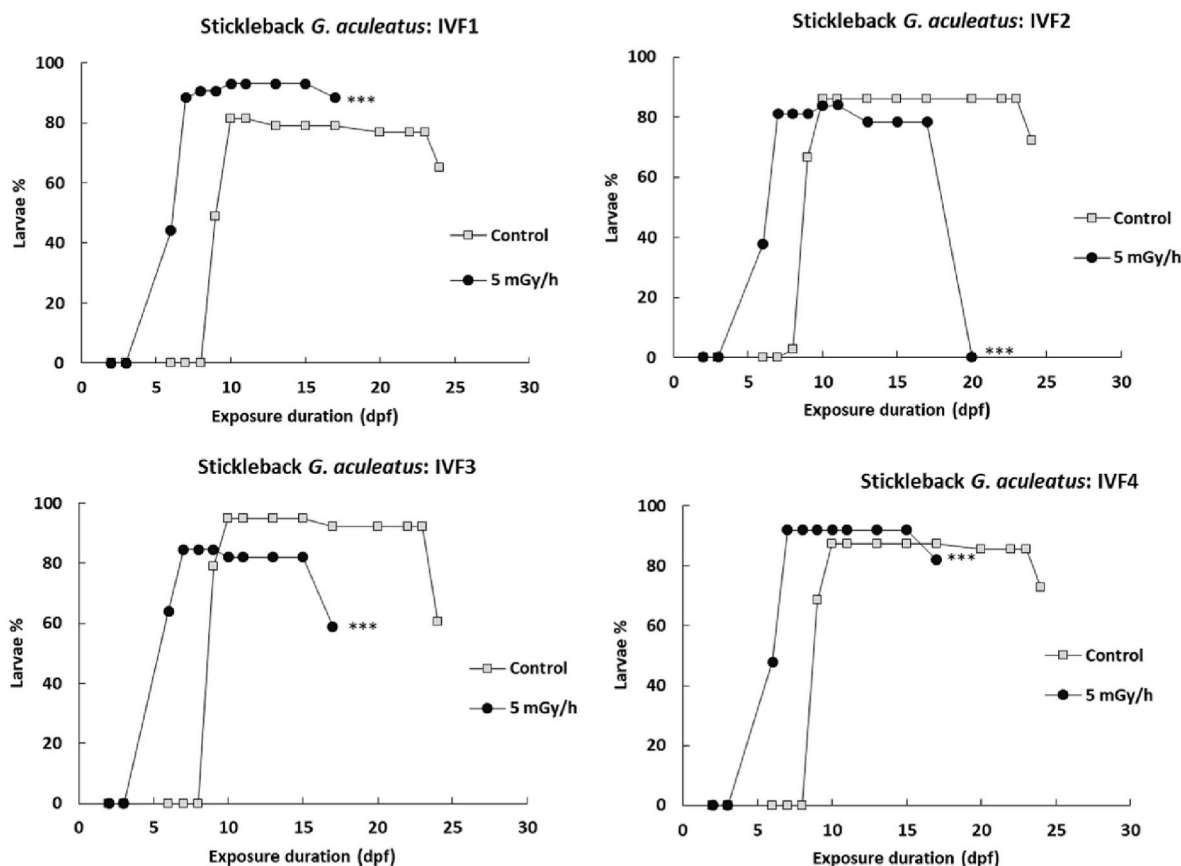


Fig. 8. Kinetics of alive larvae (%) for the four associated batches of eggs from four different IVFs for stickleback *G. aculeatus* exposed to 0, and 5 mGy h⁻¹ gamma radiation. Statistical analyses were performed using a Cox proportional-hazards Model (*p < 0.05, **p < 0.01, ***p < 0.001).

for zebrafish (Kimmel et al., 1995; Simon et al., 2011b). This process is achieved by the secretion of choriolysin but also by spontaneous movements of the developing embryos (Capiello et al., 2019; He et al., 2017). The review of literature most often shows a delay in hatching kinetic after toxic chemical exposure, probably related to the altered functioning of hatching glands (Jeziarska et al., 2009). Here, the hatching rate of wild fish species was accelerated by irradiation without an obvious dose effect, as already demonstrated for *Danio rerio* (Simon et al., 2011b) or by various stressors in brown trout, in Arctic char eggs (Wedekind and Müller, 2005) or in whitefish, vendace and stickleback (Czerkies et al., 2001; Fitzgerald et al., 2017). In trout (*O. mykiss*), an acceleration of hatching (HT₅₀) was observed in line with the increase in the temperature of the medium (5 °C–17 °C). Moreover, this acceleration depended on the stage of development exposed. The strongest effect appeared when stress was applied from the very early stages of development (Melendez and Mueller, 2021). Other irradiation conditions (accumulation of adenosine triphosphate labelled on the gamma phosphate group with ³²P, dose rate < 0.4 mGy h⁻¹) led to delayed hatching in stickleback (Lerebours et al., 2020). In trout, irradiation at later stage (48 d) confirmed the results obtained at early stage (6 hpf). These results were different from those obtained by Simon et al. (2011b) who demonstrated that irradiation for a short period (3 hpf to 24 hpf) in zebrafish does not lead to an acceleration of hatching. Other experiments were carried out at other stages of development to evaluate the existence of a critical window of irradiation.

In this study, the early hatching did not correspond to an acceleration of embryonic development because no phenotypic differences in the stages of development were observed. From a mechanistic point of view, copper (CuNPs) is known to delay hatching via inducing ROS and suppressing embryonic motility rather than the expression and secretion of hatching enzymes in *D. rerio* (Zhang et al., 2018). As gamma irradiation

is known to generate ROS, as demonstrated in zebrafish larvae (Gagnaire et al., 2015; Guirandy et al., 2019; Hu et al., 2016; Hurem et al., 2017), we hypothesize that ROS production could be responsible for stimulating fish metabolism and therefore accelerating hatching. A future molecular mechanistic approach will be necessary to understand this acceleration.

4.3. Inter-specific effects

Only in Arctic char, irradiation appeared to alter early embryonic development. A subpopulation of eggs mainly in 5 mGy h⁻¹ condition did not develop normally, remaining alive, some few being able to develop and hatch. The relatively high mortality rate compared to the other two species was due to this subpopulation of eggs with impaired development. When the subpopulation of non-eyed eggs was excluded, the survival rate of the larvae (number of larvae/number of eggs eyed at 77 d) was higher for both irradiation conditions (0.5 mGy h⁻¹: 60%, 5 mGy h⁻¹: 75%) compared to control (50%) but remained lower than the one of trout or stickleback.

The incubation period of irradiated trout eggs was greatly extended despite early hatching and abnormalities were observed. Spreading hatching over a longer period in irradiated trout could have consequences for the future of the population. The time to first hatching, HT₁₀ and HT₅₀ were shorter in irradiated conditions. Only the achievement of HT₁₀₀ was longer in the irradiated animals and contributed to this spreading of the hatching period. The malformation “larval in chorion” was not observed in control while they increase to a peak (0.5 mGy h⁻¹: 43%, 5 mGy h⁻¹: 48% of the total malformation) for the irradiation conditions. Over time, these larvae managed to extricate themselves from the chorion. This kind of malformation is related to the alteration of hatching processes. To our knowledge, this kind of alteration has not

been observed before. For example, the recent study carried out on three salmonid species did not identify such alterations (Valdebenito et al., 2021).

The reduction of survival rate of unfed stickleback old larvae after irradiation was opposite to the resistance to starvation observed in zebrafish (Simon et al., 2011b).

These results showed major interspecific differences in the toxic response.

5. Conclusion

First, no effects on mortality in these three wild species were observed despite the high dose rates and cumulative doses. For an identical dose rate, the high increase of absorbed cumulative dose between zebrafish and wild fish exposure did not lead to mortality or early development impairment. These results tend to show an interspecific homogeneity in the biological responses (mortality) and reinforce the concept of a group of reference entities (RAP) developed by ICRP. The fauna and flora protection values (DCRL) are not modified by our results. The present study demonstrates that hatching is a sensitive marker of gamma irradiation and confirms the concept of a critical irradiation window. On the other hand, sublethal effects specific to each species have been demonstrated. These interspecific sublethal phenotypic effects observed during the development of the early stages, a key stage of development, could have molecular and phenotypic repercussions on the later development stages. Finally, these results encourage us to identify the mechanisms of action and repair at the molecular scale. This step will make it possible to assess whether these mechanisms are common to these three species.

CRedit authorship contribution statement

O. Simon: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **N. Guirandy:** Writing – review & editing, Funding acquisition. **L. Dasque:** Formal analysis. **N. Dubourg:** Conceptualization. **V. Camilleri:** Formal analysis. **I. Cavalié:** Formal analysis. **C. Turiès:** Conceptualization. **A. Bado-Nilles:** Conceptualization. **L. Espinat:** Conceptualization. **E. Réalis:** Writing – review & editing, Supervision, Conceptualization. **B. Gagnaire:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

SIMON reports financial support was provided by Institute of Radiation Protection and Nuclear Safety. None If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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