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Irradiation of rainbow trout at early life stages results in trans-generational effects including the induction of a bystander effect in non-irradiated fish

Richard W. Smith [a,b,*], Colin B. Seymour [b], Richard D. Moccia [a], Carmel E. Mothersill [b]

[a] Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada
[b] Department of Medical Physics and Applied Radiation Sciences, McMaster University, Hamilton, Ontario, Canada

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ABSTRACT

The bystander effect, a non-targeted effect (NTE) of radiation, which describes the response by non-irradiated organisms to signals emitted by irradiated organisms, has been documented in a number of fish species. However transgenerational effects of radiation (including NTE) have yet to be studied in fish. Therefore rainbow trout, which were irradiated as eggs at 48 h after fertilisation, eyed eggs, yolk sac larvae or first feeders, were bred to generate a F1 generation and these F1 fish were bred to generate a F2 generation. F1 and F2 fish were swam with non-irradiated bystander fish. Media from explants of F1 eyed eggs, F1 one year old fish gill and F1 two year old fish gill and spleen samples, and F2 two year old gill and spleen samples, as well as from bystander eggs/fish, was used to treat a reporter cell line, which was then assayed for changes in cellular survival/growth. The results were complex and dependent on irradiation history, age (in the case of the F1 generation), and were tissue specific. For example, irradiation of one parent often resulted in effects not seen with irradiation of both parents. This suggests that, unlike mammals, in certain circumstances maternal and paternal irradiation may be equally important. This study also showed that trout can induce a bystander effect 2 generations after irradiation, which further emphasises the importance of the bystander effect in aquatic radiobiology. Given the complex community structure in aquatic ecosystems, these results may have significant implications for environmental radiological protection.

1. Introduction

Recent studies of non-targeted effects (NTE) of low dose ionising radiation suggest that they do occur in vivo and can be transmitted in vivo either to other organisms, or to the descendants of the irradiated organisms; examples include species as diverse as mice (Dubrova et al., 2000), daphnia (Sarapultseva and Gorski, 2013) and arabidopsis (Migicovsky and Kovalchuk, 2014). This raises the possibility that NTE may need to be considered in risk estimates of radiation exposure in the environment. However the problem with demonstrating transgenerational effects is the long-term nature of the experiments. This has meant that most research has been done using well known short lived animal models in laboratory settings, causing speculation about the real relevance of these effects in a natural environment.

As an attempt to bridge the gap to wild populations our group has conducted experiments on various fish species over the last several years to determine the effects of low dose exposures to low and high linear energy transfer (LET) radiation, including the induction of the so-called bystander effect, which describes the response of non-irradiated cells which have received signals from irradiated cells (Mothersill and Seymour, 2004).

Rainbow trout (*Oncorhynchus mykiss*) (Mothersill et al., 2006), zebrafish (*Danio rerio*) (Mothersill et al., 2007, 2012; Saroya et al., 2010) and medaka (*Oryzias latipes*) (Mothersill et al., 2009; Smith et al., 2011) have shown that, within 2–4 h of exposure, a single 0.5 Gy X-ray dose results in the production of signals, from the irradiated fish which impact non-irradiated bystander fish, which had swam with the irradiated fish, which reduce the survival of a sensitive reporter cell line. This suggests these are universal immediate/short term responses to a specific direct irradiation regimen. With a longer interval between 0.5 Gy X-ray exposure and bystander effect induction the direct irradiation and bystander effect the reporter cell response becomes less straightforward. In two year old zebrafish the signal, from the irradiated fish, which caused a reduction in reporter cell survival, was found to be attenuated within 6 h of irradiation and the effect, in the bystander

* Corresponding author at: Animal Biosciences, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 2W1.
E-mail address: rich.wilson.smith@gmail.com (R.W. Smith).

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fish, was attenuated within 12 h (Mothersill et al., 2007). However irradiation, with the same 0.5 Gy X-ray dose, at one of four early life stages, egg 48 h after fertilisation, eyed egg, yolk sac larvae (YSL) and first feeder, results in a two year legacy effect in rainbow trout, which includes the induction of a bystander effect in non-irradiated fish, (Mothersill et al., 2010). The responses of the clonogenic reporter cell line, caused by factors emitted by the irradiated fish and the bystander fish varied, depending on which early life stage was irradiated and at which age, after irradiation, the bystander effect is induced. The reporter cell effects could be pro-death or growth promoting, or completely attenuated (Mothersill et al., 2010). A similar story exists with fathead minnows (*Pimphales promelas*), injected with a single environmentally relevant dose of ^{226}Ra. There is a general pro-death response, by reporter cells treated with the medium from ^{226}Ra-injected fathead minnow tissue explants, up to 6 months after injection (Smith et al., 2013) but, depending on injection dose and the time interval after injection, the bystander effect of non-injected fathead minnows, which had swam with the injected fish, on these reporter cells, remained pro-death, became growth promoting, or was completely attenuated (Smith et al., 2013).

Unlike the situation where constant exposure to radiation results in a transgenerational effects (e.g. Buisset-Goussen et al., 2014), it is easier to overlook, or dismiss as inconsequential, the potential transgenerational impact of a single unrepeated radiation event. Therefore the fact these highly complex long term effects, following exposure to a single radiation dose, do occur in fish underlines the importance of considering the possible consequences of exposure to a single radiation dose in aquatic environmental radiological protection.

One question which has yet to be considered in the possibility of transgenerational effects of a single radiation exposure in fish. Exposure to a single radiation dose has been shown to induce transgenerational mutations and DNA damage in mice (Barber et al., 2006) and in humans accidental parental exposure to ^{137}Cs has resulted in human mutations in the F1 generation 19 years after irradiation (da Cruz et al., 2008). However, despite these examples, the widely accepted view that radiation can induce effects, such as DNA damage and genomic instability, which can then continue through a number of generations (reviewed by Kovalchuk and Baulch (2008)), is sometimes contradicted. There are reports of transgenerational genomic instability related to radiation exposure around Chernobyl (reviewed by Morgan (2003)) yet there are also claims that there is no statistically significant difference in the mutation of children of Chernobyl liquidators (Furitsu et al., 2005). In fact a recent study of the descendants of A-bomb survivors concluded that human health is not significantly affected by the transgenerational effects of radiation (Little et al., 2013). Studies on non-human/non-mammalian species are therefore required.

Therefore the aim of this study was to determine if there are transgenerational effects in fish exposed to a single radiation dose at early life stages. To do this we have built upon the previous investigation of early life stage irradiation in rainbow trout (Mothersill et al., 2010) by breeding these irradiated fish and analysing the F1 and F2 generations for pro-death or cellular growth-enhancing responses. We also set out to characterise the nature of bystander signals emitted from these progeny on non-irradiated fish which occupied the same water.

2. Materials and methods

2.1. Fish stock and breeding

All procedures used in this investigation were carried out in accordance with the animal care protocols and regulatory guidelines imposed by McMaster University Central Animal Facility and by the University of Guelph, and were specifically covered by McMaster University Animal Utilisation Protocol (AUP) 06-12-65.

The F1 and F2 trout rainbow trout were bred from a F0 experimental stock of fish, derived from fertilised eggs taken from the larger breeding stock routinely maintained by the Alma Research Station, University of Guelph. These F0 fish were exposed to a single 0.5 Gy X-ray dose at one of 4 early life stages; eggs at 48 h after fertilisation (48 h eggs), eyed eggs, yolk sac larvae (YSL) and first feeders (Mothersill et al., 2010).

All irradiations took place at McMaster University. The 48 h eggs, eyed eggs, YSL and first feeders were transported to McMaster University in insulated containers (8 °C) containing continually oxygenated water. A 0.5 Gy X-ray dose was administered using a Model 43855 A Faxitron single cabinet X-ray system (Faxitron X-ray Corporation cabinet X-ray system, Wheeling, Il, USA). This device uses a non-filtered X-ray source, delivered by 100–110 keV over 5 min (i.e. 0.1 Gy min^{-1}). Dosimetry was previously confirmed by thermoluminescence dosimetry (TLD) calibration (Mothersill et al., 2006). Approximately 500 48 h eggs and eyed eggs, and 200 YSL and first feeders were irradiated. The 48 h eggs and eyed eggs were irradiated, as a single layer, in a shallow tray containing 3.0 l water. The YSL and the first feeders were irradiated in batches of 20 in 3.0 l water. Once irradiated the eggs, YSL and first feeders were transported back to the Alma Research Station (again in insulated containers in continually oxygenated water) and then reared until sexually mature at around 2 years old.

To produce a F1 generation sperm and eggs were collected from 5 males and 5 females of each of the radiation treated groups, and also from an untreated group, derived from the same original F0 stock. The eggs and sperm from each group were pooled and then used to make a number of specific crosses: (1) male and female trout from the same original radiation treatment group were crossed, (2) male and female trout from each radiation treatment group were crossed with untreated fish and (3) male and female trout from the group irradiated as eggs 48 h after fertilisation were

Table 1
Crosses from F0 fish irradiated as 48 h eggs, eyed eggs, yolk sac larvae (YSL) and first feeders, used to produce the F1 generation.

Same parent irradiation	One parent only irradiation	48 h egg irradiation combinations
♂ 48 h egg × ♀ 48 h egg	♂ 48 h egg × ♀ untreated	♂ 48 h egg × ♀ Eyed egg
♂ Eyed egg × ♀ Eyed egg	♂ Untreated × ♀ 48 h egg	♂ Eyed egg × ♀ 48 h egg
♂ YSL × ♀ YSL	♂ Eyed egg × ♀ Untreated	♂ 48 h egg × ♀ YSL
♂ First feeder × ♀ First feeder	♂ Untreated × ♀ Eyed egg	♂ YSL × ♀ 48 h egg
	♂ YSL × ♀ Untreated	♂ 48 h egg × ♀ First feeder
	♂ Untreated × ♀ YSL	♂ First feeder × ♀ 48 h egg
	♂ First feeder × ♀ Untreated	
	♂ Untreated × ♀ First feeder	