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Short Communication

The Radiation Induced Bystander Effect: Is there Relevance for Aquaculture?

Richard W. Smith* and Richard D. Moccia

Department of Animal Biosciences, University of Guelph, Canada

***Corresponding author**

Richard W. Smith, Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Aquaculture Centre, Canada, Email: rich.wilson.smith@gmail.com

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Abstract

The radiation induced bystander effect describes the response of non-irradiated cells to transmissible signals emitted from irradiated cells. This has been shown *in vivo* in more than one species of irradiated fish swimming with non-irradiated bystander fish. Irradiation of adult fish results in a short-term bystander effect which then attenuates. However early life stage irradiation results in long term bystander effect induction, by the adults grown from irradiated eggs and larvae. This extends to a transgenerational effect, with the F1 and F2 generations of the irradiated fish being able to induce a bystander effect. Bystander effect induction also occurs between different fish species and between trophic levels. Proteomic analysis has revealed that direct irradiation results in proteomic changes that are indicative of tumorigenesis, whereas bystander effect proteomic changes suggest a protective or restorative response. The proteomic changes caused by the bystander effect also suggest that reactive oxygen species may be a component of the bystander signal. However experiments using a solid partition, between the irradiated and bystander fish, have demonstrated a physical component as well. The bystander effect is modulated by serotonin which suggests social rank may determine bystander effect induction. Overall the findings summarised in this review suggest that there may be relevance for the bystander effect in aquaculture which we propose now requires experimental attention.

INTRODUCTION; radiation in aquaculture

Possibly the most widespread source of radiation in aquaculture is dissolved radon. Radon is an inert gas resulting from the radioactive decay of radium. Three isotopes exist (^{219}Rn, ^{220}Rn and ^{222}Rn) all of which are naturally present in ground waters; e.g. [1,2]. Although streams and rivers typically contain low levels of radon, many aquaculture operations draw water from aquifers where levels can be considerably higher. The release of this radon, resulting from the necessary aeration of aquifer water, is not just a cause of concern for the working environment. Approximately 50% of the radon can remain the water used to hold the fish [3,4]. Therefore it is quite possible for cultured fish to be exposed to low-level waterborne radioactivity, particularly in hatcheries which use aquifer derived water.

The radiation-induced bystander effect

The bystander effect describes the response by completely non-irradiated cells to signals which are emitted from irradiated cells; e.g. reviewed by [5,6]. The majority of investigations have been made *in vitro*, using media transfer from irradiated to non-irradiated cell cultures [7]. However the bystander effect has been observed *in vivo*, in animals as diverse as invertebrates [8], amphibians [9] and mammals [10,11], as well as fish (reviewed here). Most significantly the bystander effect is seen in the environmentally relevant radiation range (<10 mGy), with a threshold of induction at about 2 – 3 mGy [12].

In an aquaculture environment, the relatively high stocking densities could promote bystander effect induction. However, to date, there has been little effort to understand the implications of the radiation-induced bystander effect on farmed fish. Therefore the aim of this review is to summarise some of the existing experimental work, with a view to identifying where the bystander effect could be induced, the possible implications for aquaculture and where additional research could be of value.

Induction, duration and attenuation of the radiation-induced bystander effect in fish

The bystander effect in fish has been characterised in a number of studies using a single 0.5 GyX-ray dose. Irradiated and non-irradiated bystander fish were swum together for 2h and tissue samples were collected. Media, from the resulting primary cell cultures, was then used to treat the HPV-G clonogenic reporter cell line [13]. These cells have reduced p53 gene expression and, as such, respond to anti-apoptotic or toxic signals [14].

Media from the gill, fin, spleen and kidney of irradiated and bystander rainbow trout (*Oncorhynchus mykiss*) induced a toxic response in the HPV-G cells [15]. There was a similar toxic response from the spleen of the irradiated fish but not the bystander fish [15], which could suggest there is some tissue specificity to the bystander effect. However the gills and skin of irradiated and bystander zebrafish (*Danio rerio*) [16] and the fin of irradiated and bystander medaka (*Oryziaslatipes*) [17,18] gave identical responses. Thus the radiation-induced bystander effect has been demonstrated inwidely differing fish species.

The bystander effect is not confined to adult fish. Irradiated and bystander rainbow trout eggs, yolk sac larvae and first feeders also initiated atoxic effect in the reporter cell line [19]. Furthermore two year old adults, grown from these irradiated eggs, yolk sac larvae and first feeders, and the two year old bystander trout, which swam with these fish, induced both toxic and anti-apoptotic responses, depending on which early life stage had been irradiated [19]. F1 and F2 generation fish, bred from these early life stage irradiated trout, also caused toxic or anti-apoptotic responses in bystander fish, depending on the tissue and the parental cross [20]. Irradiation at the embryonic or immediately post-embryonic stages can therefore have long term consequences. Thus, even if an aquaculture facility uses radiation free surface water, the radiological effect on any fish reared from eggs obtained from hatcheries supplied with radon contaminated ground water may extend throughout and beyond the lifetime of these irradiated fish.

The next relevant question, after bystander effect induction, is bystander signal and bystander effect attenuation. Experiments on adult zebrafish have shown that 12h after irradiation the irradiated fish did not induce a bystander effect, suggesting the signal was no longer emitted and, in bystander fish, within 6h of being induced the bystander effect itself was lost [16]. This would suggest that radiation exposure and bystander effect induction in adult fish may have less long term significance than at the embryonic and juvenile life stages (although more species do need to be evaluated). Similarly water flow or the ratio of irradiated fish biomass to water volume would presumably dilute or eliminate the bystander signal. Although no studies have been carried out *in vivo* the bystander effect can be eliminated *in vitro* by the dilution of cell culture media [21]. The water dynamics of an aquaculture facility are therefore highly likely to determine the extent of bystander effect induction.

The majority of aquaculture operations involve a single species. However farmed fish are cultured with other species; e.g. the use of wrasse to control sea lice in Atlantic salmon (*Salmo salar*) [22-24] and the integrated multi-trophic aquaculture of black rockfish (*Sebastes schlegeli*), sea cucumber (*Apostichopus japonicas*) and oyster (*Crassostrea gigas*) for the potential nutritional exploitation of caged fish waste [25]. X-ray exposure, combined with the use of the HPV-G reporter cell line, has shown that an interspecies (zebrafish and medaka) and inter-phyla / trophic level (rainbow trout and California blackworm, *Lumbriculus variegatus*) bystander effect can be induced [26]. Based on these findings bystander effect induction in mixed species aquaculture has to be considered a possibility.

Another consideration is a more complex radiation exposure scenario; e.g. multiple doses or the influence of an additional stressor. Chronic exposure (0.03 – 5.88 Gy over a 264 day period), prior to a 0.5Gy acute X-ray dose, changes the toxic response of HPV-G reporter cells, from both the irradiated and the bystander fish, to an anti-apoptotic response [18]. Water quality is obviously of paramount concern for aquaculture and the bystander effect in trout can be rendered more toxic by acute exposure to waterborne aluminium prior to irradiation [27]. Similarly the bystander induced by 2 year old adult trout, which had irradiated as eggs, larvae or first feeders, can also be modified by acute exposure to waterborne aluminium [28].

Although the studies summarised above employed X-rays, radium (the source of radon) is the more likely means of radiation exposure in aquaculture. A single radium dose, up to 2.1 mBq (i.e. environmentally relevant [29], can induce a bystander effect in fathead minnows up to 6 months after irradiation [30]. Other possible means of irradiation cannot be ignored. For example; the use of effluent cooling water from nuclear power plants, has been considered as a viable warm water option [31] and, given that ultra-violet (UV)-B radiation, from high altitude aquaculture, does compromise antibody production in tilapia (*Oreochromis mossambicus*) [32], the possibility of a UV-induced bystander effects also exists.

It must be conceded that not all radiation induces a bystander effect. Neutron (86.8 +/- 4.3 mGy, plus 13.9 +/- 0.7mGy of associated gamma radiation) exposure of zebrafish resulted in a HPV-G cell toxic response but there was no bystander effect in fish which swam with the irradiated fish [33].

Proteomic responses to radiation and the radiation induced bystander effect in fish

A recent review [34] has illustrated the potential value of proteomic analysis as a means of fully understanding the bystander effect. In rainbow trout gills a single 0.5 Gy X-ray dose increased cancer associated annexin II (e.g. reviewed by [35], while the bystander, induced by these irradiated fish, effect resulted in the up regulation of haemopexin-like protein, rho GDP dissociation inhibitor and pyruvate dehydrogenase; i.e. proteins which could protect the gill against reactive oxygen species, maintain epithelial polarity and prevent lactate acidosis, respectively [36]. A similar study on irradiated medaka gills revealed the up regulation of annexin max 3 (a similar cancer associated protein to annexin II), but also the downregulation of annexin A4, and the upregulation of creatine kinase and lactate dehydrogenase in irradiated fish [37]. These are indicators of an apoptotic response to radiation induced tumourigenesis [37]. In contrast the bystander effect resulted in the upregulation of warm-temperature acclimation related 65-kDa protein [37], a protein which is has equivalent protective properties to haemopexin [38]. Thus, although there have been very few studies on the bystander effect proteomics in fish, the evidence collected so far suggests that the bystander effect could be possibly beneficial.

The bystander effect signalling mechanism

As stated above proteomic analysis has suggested that reactive oxygen species are a likely component of the bystander signal. However experiments using irradiated and bystander zebrafish separated by a solid barrier has also indicated a physical component to the bystander signal, possibly weakly acoustic or electromagnetic in nature [39]. The relevance of this finding to aquaculture is that induction of the bystander effect may still occur even where partitioning of the rearing vessels physically separates the irradiated fish from the non-irradiated fish.

Salmonids form social hierarchies and there is a wealth of evidence regarding social rank, aggression, food consumption and growth; e.g. [40-42]. The position of any individual in the social structure is based on brain serotonin (e.g. [43,44]); lower serotonin levels equate to dominant fish and higher serotonin levels equate to submissive fish. Experiments *in vitro* have established a threshold level of serotonin, below which there is no bystander effect induction [45] and have shown that serotonin inhibitors modulate [46] or block [47] the bystander effect. This has been confirmed *in vivo*, in zebrafish, where the injection of the serotonin inhibitor reserpine prevented the toxic response on HPV-G reporter cells [48]. A radiation induced bystander effect may therefore have a particular relevance to salmonid culture by being more prevalent in subordinate fish.

CONCLUSION

The existence of a radiation induced bystander effect has been convincingly demonstrated in fish. Based on the findings reviewed here, we propose the question of the bystander effect in aquaculture should now be investigated. The radiological history of the fish stock determines whether there is the potential for the bystander effect to be induced and the design and operation of the facility, in particular water quality and flow / turnover rate most likely determines whether or not induction actually occurs and whether this a is short or long term effect. If induction does occur, in the case of salmonid species, the implications of social hierarchy need to be specifically addressed. The fact that there is a direct relationship between bystander effect induction and social rank / serotonin levels gives cause for concern that an identifiable proportion of a cultured salmonid stock may be particularly susceptible to the bystander effect. At present it is not known if this means overall production is compromised; i.e. has economic implications. Of equal importance, from the economic standpoint, is whether public opinion is unfavourably altered by the idea that farmed fish may show responses to waterborne radiation which are not fully understood and are not covered by current radio-protective legislation. Finally the question exists as to whether the bystander effect results in a beneficial or protective response at the molecular level and thus improves the overall fitness of cultured fish. This is clearly a controversial concept, which could also affect public perception of aquaculture and which also applies to mixed species aquaculture, since signalling between species in natural ecosystems is considered beneficial [49].

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