A Preliminary Assessment of Radiation Effects on American Flagfish

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A PhD Level Submission Summary

In order to add to the knowledge base of radiation effects on non-human biota, it is important to define benchmark values for different species. An experimental set-up was designed to investigate effects from irradiation with Cs-137 to American Flagfish. Preliminary experiments to assess the suitability of the methodology were conducted by exposing Flagfish eggs to 44 h of ionizing radiation. The subsequent observation of the developing fry showed no effect on hatching. However, the mortality and observed vertebral malformations were increased with increasing absorbed dose which is suspected to be a result of developmental defects in the embryonic stage.

1. Introduction

With growing interest in environmental protection, the need has risen to investigate the effects of radiation and the resulting radiation damage to components of the natural environment. In the past the assumption was made, that “if man is adequately protected, then other living things, are also likely to be protected” [1]

Consequently a methodology is needed to evaluate the effects of radiation for the natural environment independently and also in the absence of human components. This includes approaches of calculating the magnitude of the dose absorbed by individual organisms as well as assessing the impact and resulting risk to animal populations. Similar to the methodology used in human radiation protection a numerical guidance is being developed which led to the introduction of a set of reference animals and plants following the concept of the reference man [2,3].

One of the challenges this approach is facing is the identification of biological endpoints for different species in the relevant ecosystems. Endpoints for non-human biota can range from mortality of the individual to fertility and thus population fitness. The focus of this work lies with the analysis of aquatic freshwater environments. These highly diverse and complex systems are of grave importance due to their large influence on local and global ecosystems.

2. The Research Organism American Flagfish

Besides developing modelling approaches to address the problem of direct dose measurement, there is a great research need to gather data about the reference animals and plants as well as identifying related research organisms. Those are selected based on practicability considerations and their suitability for research, like robustness to changes in the environment and a short maturation time. Model organisms are used to decouple the system to be studied from its environment in order to isolate the effects of radiation and observe specific reference and benchmark values of radiation effects. Concerning freshwater environments, one of the reference animals is the trout. Currently, the Medaka (*Oryzias latipes)* is often chosen as the main research organism for radiobiology research. It

has been extensively used as a model organism for toxicological research and it has a well studied physiology.

We investigated the American Flagfish (*Jordanella Floridae)* as a possible additional research animal for radiation effects experiments, as well as to add to the knowledge base of radiation effects to aquatic biota. The Flagfish is a freshwater fish that can be found in environments with wide variation of temperatures [4] and has been proven to be an excellent test species for laboratory studies. This is mainly because of their ease of breading and short life cycle of approximately 90-120 days. They also exhibit distinct behavioural patterns, especially in paternal care that influences the survival of the species [5].

From irradiation studies with Medaka a variety of effects has been observed. The extrapolated LD50/20 for Cs137 is reported to be approximately 25 Gy [6] and a decrease in hatchability was observed depending on the developmental stage during irradiation. Additionally, vertebral malformations were observed starting from 4 Gy as well as a decline of radiation damage with temperature and fractionated irradiation. [7,8]

3. Experimental Design

In order to establish the American Flagfish as a research organism and adding to the data of radiation effects on aquatic biota for a construction of the reference animals, the observations on Medaka or the deviation from them have to be investigated.

Based on the knowledge obtained from irradiation of Medaka, an experiment was designed with the capability to determine the effects of ionizing radiation on the hatchability of Flagfish embryos (Figure 1) as well as experimentally establish an LD50/30 for Cs137.

All work was done in compliance with the Animal Usage Protocol that was submitted and accepted by the Animal Care Committee at UOIT as well as with the regulations put in place by the Ontario Ministry of Agriculture, Food and Rural Affairs. The Three R's of humane animal experimentation (reduction, refinement, replacement) were achieved by careful experimental design geared towards using as few animals as possible while maintaining statistically significant results.

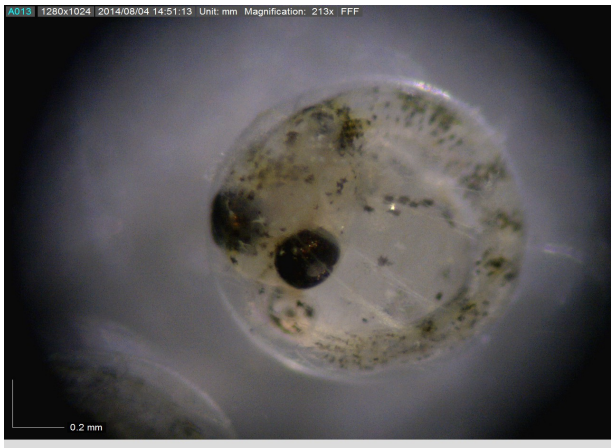

Figure 1 Healthy Flagfish egg at three days post fertilization as used in the irradiation experiments.

3.1 Procedures

Flagfish eggs were collected one day after fertilization in collaboration with the Tier-I Research Chair in Aquatic Toxicology at UOIT (Figure 1). Subsequently they were placed 44 hours in a Cs137 beam (Hopewell G10 gamma beam irradiator). During the embryonic stage and after hatching the Flagfish were monitored in a controlled environment at 24-25 degrees Celsius water temperature until the fry had absorbed the yolk sack. They were observed daily through an optical microscope to assess their fittness and track possible malformations. Moribund fry were removed and accounted for.

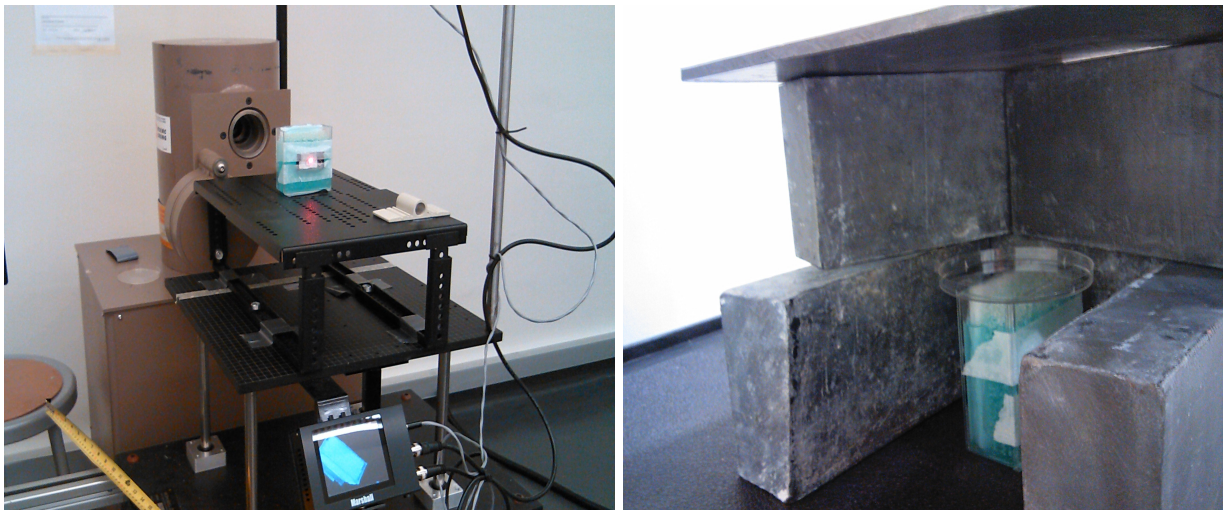

Figure 2 The experimental set-up for the irradiated and the control group.

3.2 Experimental Set-Up

The irradiation set-up can be seen in Figure 2. The container with the Flagfish eggs was placed at calculated distances from the irradiator to achieve a variety dose rates and as a result total absorbed doses of 3, 7, 11, 13 and 17 Gy. Dosimetry was done with a set of Cesium calibrated Landauer Optically Stimulated Luminescence Dosimenters. The control group was kept in the same room behind a concrete wall and lead shielding. Monitoring through OSL dosimeters ensured that the shielding was sufficient.

4. Preliminary Results

4.1 Hatchability and Mortality

Several observations were made in the maturation time of 10 days post fertilization. No effect on hatchability could be observed. However, a minimal increase in embryo malformations could be noted with increasing dose (Figure 3). As expected the mortality increased when the total absorbed dose was increased. At absorbed dose of 13 Gy the mortality was observed to be approximately 40%.

4.2 Vertebral Malformations

One reoccurring malformation that became apparent at high dose rates after hatching but before absorption of the yolk sack is a bending of the spine. This vertebral malformation was mainly observed in the region behind the head of the Flagfish fry, as can be seen in Figure 4. As of now it is unclear if that malformation leads to increased mortality or if it is hereditary. However it is very similar to the effects observed in Medaka [8].

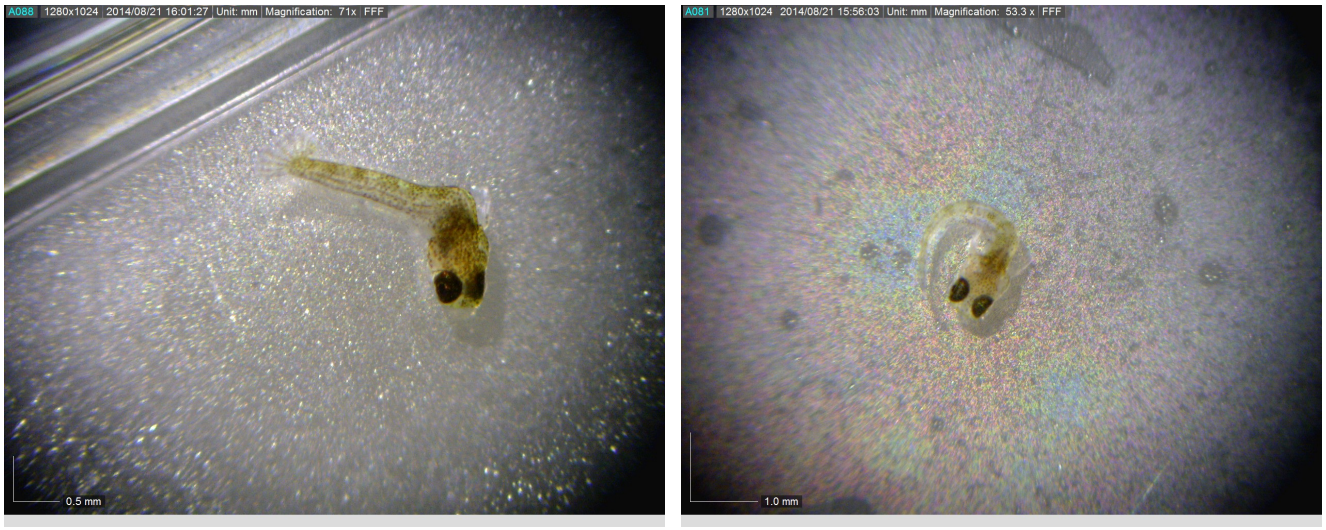

Figure 3 Vertebral Malformations of American Flagfish fry.

5. Future Work

To further expand the results of this preliminary study, a multitude of experiments are planned. The first priority is conducting an experiment and monitoring the fry for 30 days in order to measure the LD50/30. Additionally an investigation of dose rate effects as well as the effects of fractionated exposure is planned. In the future generation spanning experiments will help to determine whether the observed effects are hereditary. American Flagfish are ideal for that purpose due to their short maturation time. When a thorough understanding of the research organism is reached, an expansion to other biota native to Canada, will contribute even further to our understanding of radiation effects to non-human biota.

6. Conclusion

Summing up, the experiment demonstrated the capability of the set-up to conduct radiobiology experiments with living organisms with less that 10% mortality in the control group. When exposing American Flagfish eggs to Cs 137 no effect on hatchability could be observed, although there are suspected developmental defects that are not preventing hatching in the embryonic stage but lead to a

subsequent higher mortality. Along with the observed vertebral malformation, this effect has an impact on the long term survival. It will still need to be determined if it is hereditary.

In conclusion the definition of set values for radiation damage, like hatchability and LD50/30 for various organisms, is an important contribution to the knowledge base of radiation effects on organisms other than humans. Those findings are crucial when determining the properties of reference animals and plants. They thus lead to a better understanding of radiation effects and provide scientific reasoning for legislations for the sufficient protection not only of men but also of the environment.

7. References

[1] CRP, 1977. “Recommendations of the ICRP.” ICRP Publication 26. Ann. ICRP 1 (3).

[2] R.J. Pentreath, 2009, “Radioecology, radiobiology, and radiological protection: frameworks and fractures”, Journal of Environmental Radioactivity, Vol. 100, pp. 1019-1026.

[3] ICRP, 2008. “Environmental Protection - the Concept and Use of Reference Animals and Plants.” ICRP Publication 108. Ann. ICRP 38 (4-6).

[4] Foster, N. R., Cairns, J., and Kaesler, R. L., 1969. The flagfish, Jordanella floridae, as a laboratory animal for behavioral bioassay studies. Proceedings of the National Academy of Science Philadelphia, Vol. 121, pp. 129-152

[5] Mertz, J. C., and Barlow, G. W. ,1966. On the Reproductive Behavior of Jordanella floridae (Pisces: Cyprinodontidae) with Special Reference to a Quantitative Analysis of Parental Fanning. Z. Tierpsychol., Vol. 23, pp. 537-554.

[6] W.W. Kuhne et al, 2009 “Biological effects of high-energy neutrons measured in vivo using a vertebrate model”, Radiat. Res., Vol. 172, Iss. 4, 2009, pp. 473-480.

[7] Etoh H. and Egami N., 1967, “Damage accumulation and recovery in the fish Oryzias latipes exposed to fractionated or protracted radiation at different temperatures”, Rad. Res., Vol. 32, Iss. 4, 1967, pp. 884-891.

[8] Etoh H. and Hyodo-Taguchi Y. 1993, “Vertebral malformations in Medaka (teleost fish) after exposure to tritiated water in the embryonic stage.”, Rad. Res., Vol. 135, Iss. 3, 1993, pp. 400-404.