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Journal of Environmental Radioactivity 74 (2004) 43–55

JOURNAL OF
ENVIRONMENTAL
RADIOACTIVITY

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Low Dose Rate Irradiation Facility: initial study on chronic exposures to medaka

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Abstract

Uncertainties associated with the effects from chronic low-level exposures to radiation prompted us to construct a Low Dose Rate Irradiation Facility (LoDIF). The facility was designed specifically to test the appropriateness of the 10 mGy d⁻¹ guideline often espoused as acceptable for protection of aquatic biota from ionizing radiation. Scientists at the 0.4 ha facility use 40 outdoor mesocosms and ¹³⁷Cs irradiators of three different source strengths to research the effects of chronic low-level irradiation at different levels of biological organization. A description of the facility is included along with results from a pilot study in which Japanese medaka (a small fish native to Asia) were chronically irradiated at the highest dose rate possible within the facility (350 ± 150 mGy d⁻¹). Irradiated fish produced fewer eggs per day ($p = 0.03$); had a lower percentage of viable eggs ($p = 0.04$), and produced a lower percentage of hatchlings ($p = 0.05$). Although these data are not surprising based on the relatively high dose rates, they are important to future work at the LoDIF because they confirm the utility of our chosen model organism for detecting population-level responses, and they illustrate the statistical power achieved from using replicated mesocosms, in that statistical significance was achieved with few replicates per treatment. Future directions for the LoDIF are presented, as well as an invitation for interested researchers to participate in our studies.

Published by Elsevier Ltd.

Keywords: Biota dose; Low dose; Chronic irradiation; Radiological risks; Dose limit

1. Introduction

The criteria for protecting biota from ionizing radiation are currently being scrutinized by numerous national and international organizations (Stone, 2002).

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Questions arise largely because the long-standing paradigm for protecting the environment from ionizing radiation adopts an anthropocentric approach, and is unlike how any other form of pollutant is managed. The paradigm states that:

Although the principal objective of radiation protection is the achievement and maintenance of appropriately safe conditions for activities involving human exposure, the level of safety required for the protection of all human individuals is thought likely to be adequate to protect other species, although not necessarily individual members of those species. The Commission therefore believes that if man is adequately protected then other living things are also likely to be sufficiently protected (ICRP, 1977).

There is some rationale to this paradigm, as recently reviewed by Hinton and Whicker (2003), but its presence is in part why there are currently no internationally agreed upon criteria or policies that explicitly address protection of the environment from ionizing radiation, although many international agreements and statutes call for protection against pollution (ICRP, 2002).

Over a decade ago, the International Atomic Energy Agency (IAEA, 1992) conducted a literature review of radiation effects to biota with the intent of assessing the paradigm. The IAEA (1992) suggested that reproduction was a more sensitive endpoint than mortality, and offered, as a general guideline, that if maximally exposed individuals receive dose rates less than 10 mGy d^{-1} then populations of aquatic organisms will be protected. The US Department of Energy (DOE) recently adopted 10 mGy d^{-1} as a criterion in their technical standard for Radiation Protection of the Public and the Environment (DOE, 2002). However, more recent reviews suggest that reproduction in fish can be negatively affected at lower dose rates ($2\text{--}5 \text{ mGy d}^{-1}$; Sazykina and Kryshev, 2003), and that threshold levels for the first negative changes in immune system responses occur at only $0.5\text{--}1 \text{ mGy d}^{-1}$. The Canadian Nuclear Safety Commission, using a traditional ecological risk approach, has also used a more conservative dose rate than the DOE by establishing an Estimated No Effect Value for fish of 0.5 mGy d^{-1} (Bird et al., 2003). Thus, agreement has not yet been reached as to what dose rate level is acceptable for biota.

Even though the paradigm may eventually be supported, the existing database is inadequate to do so, particularly for chronic, low-level exposures or in situations with multiple stressors. Actually, few studies exist that are directly relevant to understanding the responses of plant and animal populations to radionuclides in their natural environments. Most studies have emphasized individual rather than population responses; mortality rather than reproduction; acute rather than chronic irradiation; external gamma irradiation rather than internal contamination; single contaminants rather than mixtures; and primary rather than secondary effects (Whicker and Hinton, 1996).

There is not even agreement as to what endpoint should be measured to quantify an environmental effect following exposure to radiation (Hinton, 1998), particularly with the ever-growing enhanced abilities to detect damage at the molecular

and cellular levels. The latter is important because the recently proposed ICRP (2002) framework for protecting biota from ionizing radiation includes molecular effects (e.g. scorable DNA damage) as endpoints. Likewise, a major research consortium (FASSET: Framework for Assessment of Environment Impact), funded by the European Union, has also recommended that cytogenetic damage be used as an effect endpoint (FASSET, 2002). However, a quantifiable connection between changes in molecular/cellular endpoints and declines in populations due to irradiation has not yet been made. The inclusion of sub-lethal endpoints begs for a linkage to be established between molecular effects and those observed in individuals and populations.

The uncertainties associated with the effects from chronic low-level exposures to radiation prompted us to construct a Low Dose Rate Irradiation Facility (LoDIF) specifically to test the appropriateness of the 10 mGy d⁻¹ guideline, and to conduct research on the effects of chronic low-level irradiation at different levels of biological organization. That is, how much molecular damage is required before effects are observed in individuals, and in turn how much is required before population effects are observed? In this first paper we describe the LoDIF, report on a pilot experiment designed to test for population level effects in fish exposed to the highest dose rate possible in our facility, and issue an invitation for interested researchers to collaborate with us in our quest to better understand the effects of chronic, low-level irradiation.

2. Materials and methods

2.1. Low Dose Rate Irradiation Facility

LoDIF is an outdoor, gamma-irradiation array consisting of 40 fiberglass, open-air tanks designed to house a variety of aquatic organisms. Each tank, or mesocosm, is parabolic in shape, 2.4 m in diameter and holds approximately 965 L of water with a maximum depth of 41 cm (Fig. 1). The mesocosms are of a flow-through design and receive water from a nearby lake at rates that were adjusted to about 1L min⁻¹. For randomized block experimental designs, the facility is arranged into eight 6.5 m×10.5 m blocks, with each block containing five mesocosms (Fig. 2). The facility covers approximately 0.4 ha in area.

The mesocosms allow manipulative experiments to be conducted on whole organisms in conditions more natural than laboratory settings, similar to that which has been successfully used in ecology and population biology (Wilbur, 1987; Rowe and Dunson, 1994). The use of mesocosms occupies a middle position between realistic but uncontrolled field experiments, and highly controlled yet unrealistic laboratory experiments. Mesocosms allow the researcher to apply specific treatments in a more controlled environment than large-scale field tests. Compared to field tests, the greatest advantage of using mesocosms is the ease of replicating treatments such that powerful statistical methods (e.g. ANOVA) can be used (Rowe and Dunson, 1994).

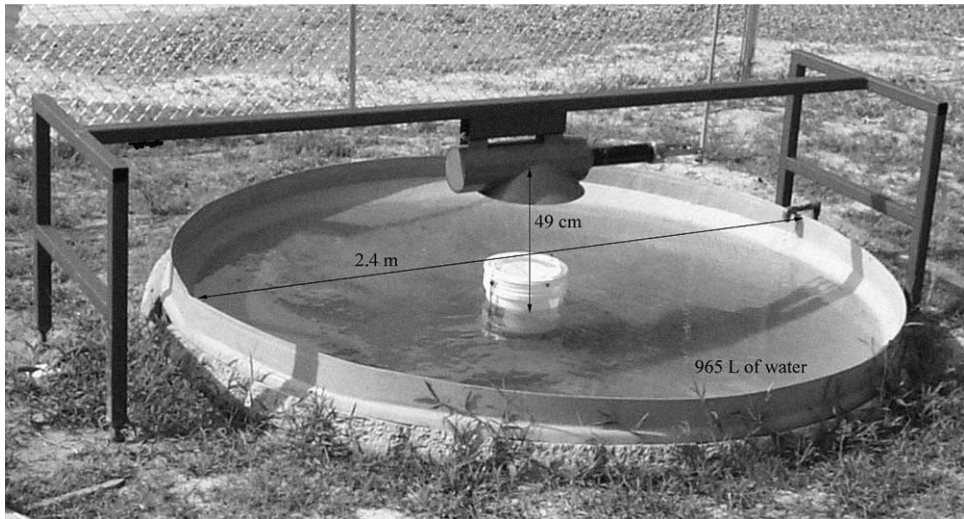


Fig. 1. Photo of a single mesocosm, showing the dimensions of the tank and a ^{137}Cs irradiator with collimated shielding stationed above it. The Low Dose Rate Irradiation Facility (LoDIF) has 40 such tanks installed over a 0.4 ha area.

Perhaps the most unique feature of the LoDIF is the ability to deliver low dose rate radiation treatments to any of the 40 mesocosms. Specially designed irradiators are mounted on steel frames and placed over each mesocosm. Each irradiator contains either a 0.74, 7.4 or 74.0 MBq sealed ^{137}Cs source within a lead container collimated to deliver an exposure to animals residing in the tank below. The distance from a ^{137}Cs source to the surface of the water in a mesocosm is 49 cm (Fig. 1). The 7.4 MBq source strength and associated geometry was designed to deliver a mean dose rate of approximately 10 mGy d^{-1} to organisms residing within the mesocosms. The other two source strengths give mean dose rates that are factors of 10 less than, and greater than 10 mGy d^{-1} . Thus, the facility was designed specifically to determine if populations of aquatic organisms are truly protected by what is currently thought to be appropriate dose rates.

Dose rates within a mesocosm are greatest directly under the center of a ^{137}Cs source, and decrease 70-fold at the horizontal edge of the mesocosm. In the center axis, directly under the source, dose rates drop by a factor of 5 from the surface of the water to the bottom of the mesocosms, due to distance and shielding provided by the water. This heterogeneity can be used advantageously by subdividing the mesocosms into two distinct areas, each with a more homogeneous dose distribution. A higher dose rate section can be partitioned directly under the irradiator, while the mesocosm's isolated peripheral area comprises a lower dose rate section. This allows two or more experiments to be conducted simultaneously within each of the 40 mesocosms. Alternatively, organisms can be allowed to utilize an entire mesocosm and an averaging of the exposure used to estimate dose. Thermoluminescent

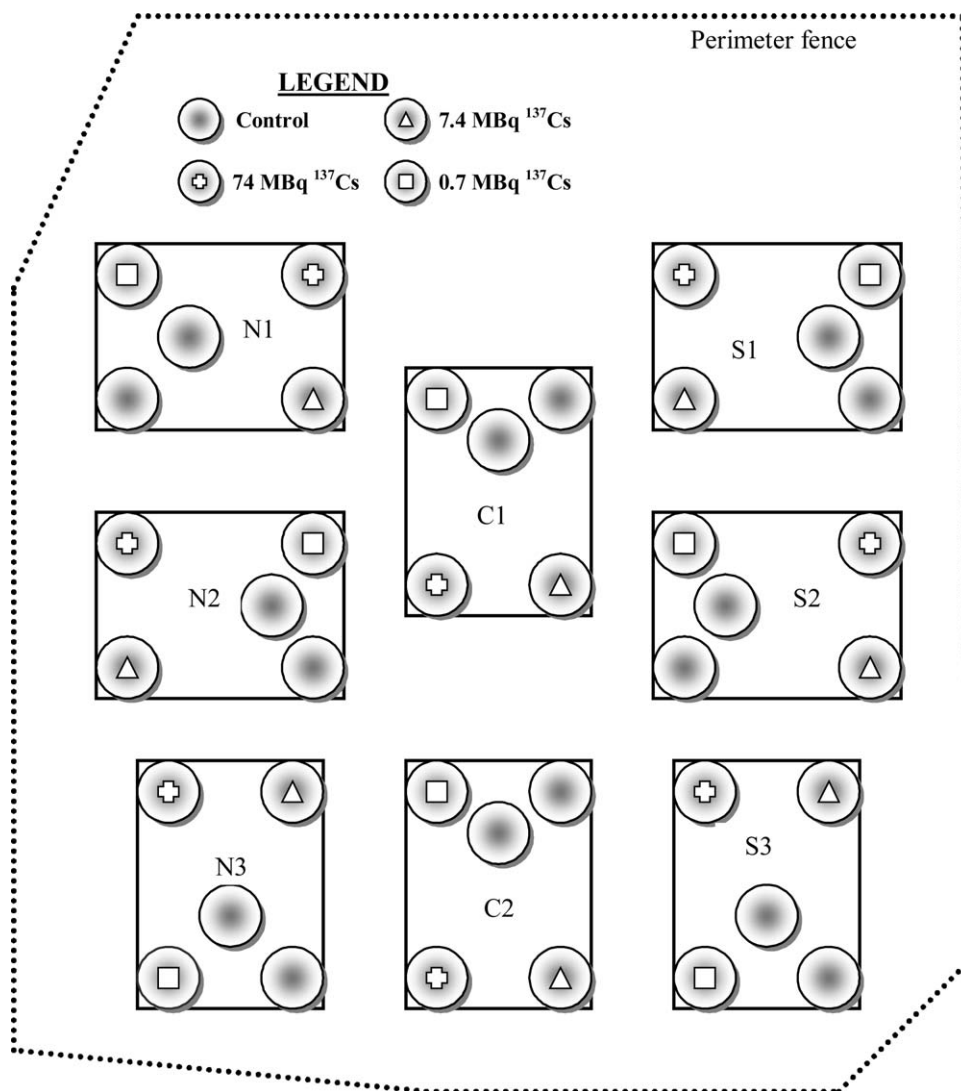


Fig. 2. Schematic diagram of the Low Dose Rate Irradiation Facility (LoDIF) showing eight pads, each containing five mesocosms (two non-irradiated controls, and three with ¹³⁷Cs irradiators of 0.74, 7.4, and 74 MBq source strength). Each mesocosm is designed to house aquatic organisms in a flow-through design.

dosimeters can be placed within the exposure field to obtain mean dose rate estimates, or directly on individual animals for the most precise data.

2.2. Model organism

We have selected a species of small fish, the Japanese medaka (*Oryzias latipes*), as our model organism. Medaka is one of the most widely used fish in comparative

mutagenesis and carcinogenesis studies. Shimada and Shima (1998) showed that the mutational response of the medaka male germ cell was comparable with that of the mouse, and proposed that medaka be a new non-mammalian model system for risk assessment of environmental mutagens. The genetics, developmental biology, embryology, and specific developmental stages of medaka have been extensively characterized (Yamamoto, 1975). The use of medaka in biomedical and environmental research, especially as a carcinogenesis model related to identifying and predicting human health effects from toxicant exposure, has received considerable attention (Wittbrodt et al., 2002; Winn et al., 2000; Metcalfe, 1989; Hendricks, 1982). The species has numerous advantages for experimental laboratory and field studies including: small size (about 2.5 cm in length); a short generation time of 6–8 weeks; and a prolific capacity to reproduce (6–30 eggs d^{-1} with up to 3000 eggs per female in a single breeding cycle).

2.3. Experimental design of pilot study

The pilot experiment compared four population endpoints obtained from fish exposed to four different treatments: (1) gamma irradiation from the highest strength source in the LoDIF; (2) a cadmium contaminated diet; (3) a cadmium diet plus gamma irradiation; and (4) non-irradiated controls fed uncontaminated diets. The endpoints analyzed were: (1) the mean number of eggs produced per female per day; (2) the percent of eggs that were viable; (3) the percent of viable eggs that hatched; and (4) the percent survival of hatchlings, 10 d post hatching. Approximately 17 subadult medaka (approximately 60 d old) were placed in each mesocosm. From a statistical analysis perspective, the experimental units were individual mesocosms, rather than individual fish, and each treatment (mesocosm) was replicated four times, for a total of 16 mesocosms.

By enclosing the fish within a partitioned area directly under the highest strength irradiators (74 MBq of ^{137}Cs), the highest level of gamma exposure was obtained. Dose rates were measured with lithium fluoride TLDs and found to vary between $558 \pm 88 \text{ mGy d}^{-1}$ at the water's surface and $140 \pm 11 \text{ mGy d}^{-1}$ at the bottom of the tanks. Thus fish were exposed to mean dose rates of $350 \pm 150 \text{ mGy d}^{-1}$. Irradiation of the fish began on 3 July. Mean weights and lengths for groups of fish within each mesocosm were taken prior to the start of the experiment and again on 6 August when the irradiators were turned off. A mean condition factor index (CF; Anderson and Gutreuter, 1984) was estimated for each group as:

$$\text{CF} = \frac{\text{mean fish mass (g)}}{\text{mean fish length (cm)}^3} \times 100 \quad (1)$$

Irradiators were on for a total of 28 d, resulting in a total mean dose of $10 \pm 5 \text{ Gy}$, depending on where in the water column the fish resided. Researcher access to the LoDIF was restricted by Health Physics personnel to 1 day per week. Thus, while in the mesocosms, fish were fed dried brine shrimp, twice daily, from automatic feeders. At cessation of irradiation, three females and two males were randomly chosen from each mesocosm and transferred as a group to separate breeding tanks inside

the laboratory, where water temperature was controlled to ideal breeding conditions (26 °C). Natural lighting was provided through laboratory windows. Breeding fish within the laboratory were fed an abundance of brine shrimp two to three times a day. Following an 8-d period to acclimate the fish to the new diet and laboratory conditions, eggs were collected each morning for 3 d (14–16 August). Eggs were incubated in a saline solution and checked daily for infertile eggs that were promptly removed. Hatchlings were removed, maintained in saline solutions, and fed brine shrimp.

3. Results

Problems with the automatic feeders resulted in fish inadvertently being on a minimal maintenance diet during the treatment period. This was evident from the negative condition factor indices (Eq. (1)), and poor weight gains observed in each mesocosm (Table 1). Indeed, mean weights of fish from six mesocosms declined during the last three weeks of the experiment. Weight gains and associated health of the fish were crucial to our endpoints in this experiment, evidenced by the correlation ($r^2 = 0.66$) in eggs produced per female to the mean change in mass of fish during the course of the experiment (Fig. 3). Reduced resource allocation to reproductive effort, during periods of declining nutritional status, has been reported many times for a wide variety of organisms (e.g. Coward and Bromage, 1999). Inadequate nutrition can be as great a stressor on egg production as xenobiotics (Patyna et al., 1999). Thus, change in fish mass was a confounding variable to the irradiation treatments. As discussed by Davis et al. (2002), these correlations also

Table 1

Mean change in mass and condition factor (CF; see Eq. (1)) experienced by the fish during the experiment due to inadvertent problems with automatic feeders. The typical mass of fish at the end of the experiment was about 0.2 g, thus the mean changes in mass shown here can represent a sizable fraction of the animal's mass

Treatment	Mean mass change (g)	Mean change in CF
Control	0.056	– 0.44
Control	– 0.107	– 1.36
Control	0.004	– 0.58
Control	– 0.032	– 0.99
Irradiated	– 0.072	– 0.93
Irradiated	0.059	– 0.20
Irradiated	0.014	– 0.57
Irradiated	0.057	– 0.22
Cd diet	0.108	– 0.10
Cd diet	0.036	– 0.46
Cd diet	– 0.003	– 0.61
Cd plus irradi.	0.058	– 0.26
Cd plus irradi.	0.021	– 0.82
Cd plus irradi.	– 0.009	– 0.37
Cd plus irradi.	– 0.039	– 0.94

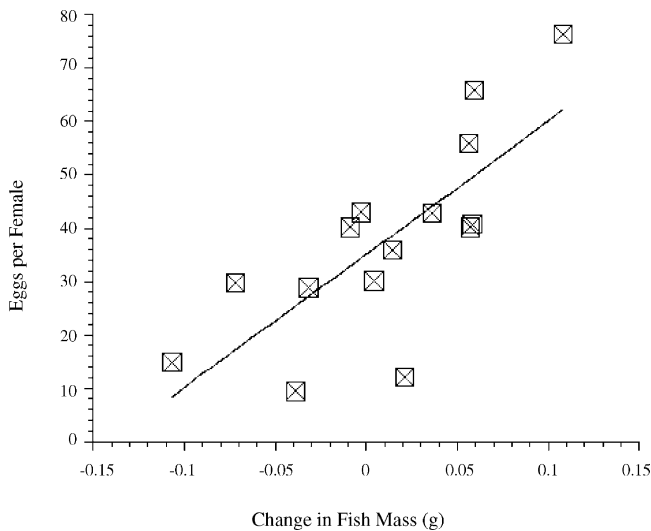


Fig. 3. Correlation between the mean number of eggs produced per female and the mean change in fish mass during the course of the experiment ($r^2 = 0.666$). The mean fish mass at the start of irradiation was 0.19 g.

highlight the potential problem of correctly interpreting results when animal husbandry practices alter the endpoints of interest. Therefore, we controlled for the unplanned loss in fish mass by using condition factor (Eq. (1)) as a covariate in our analysis of variance (ANCOVA).

Inadequate delivery of food also negated the dietary cadmium treatments. Analysis of fish at the end of the experiment revealed that there was no difference ($p = 0.16$) in Cd body burdens on the contaminated diets and those that were not. ANCOVA also indicated that none of the four endpoints differed significantly for control animals vs. those on the cadmium diet, or for irradiated animals vs. those on cadmium diet plus irradiation (Table 2). Therefore, we elected to pool irradiated treatments together (i.e. gamma plus Cd diet treatment, $n = 4$, was pooled with the gamma only treatment, $n = 4$), and to pool non-irradiated treatments together (i.e. Cd dietary treatment, $n = 3$, was pooled with controls, $n = 4$). Our analysis was thus reduced to a single factor comparison of irradiated ($n = 8$) to non-irradiated animals ($n = 7$).

Significant differences between irradiated and non-irradiated treatments were observed for three of the endpoints measured (Fig. 4). ANCOVA indicated that when compared to non-irradiated animals, irradiated fish:

- produced fewer eggs per female per day ($p = 0.03$),
- had a lower percentage of viable eggs ($p = 0.04$),
- had a lower percentage of hatchlings produced from the viable eggs ($p = 0.05$),
- tended to have a reduced survival rate among hatchlings, 10 d post-hatching, ($p = 0.11$).

Table 2

Probability values from ANCOVA tests among treatments. These data, in conjunction with Cd tissue analyses of the fish, motivated us to pool treatments and only analyze irradiated vs. non-irradiated animals (see text for explanation)

Treatments compared	ANCOVA probability values for specific endpoints				
	Total number of eggs	Eggs per Female	% viable eggs	% that hatched	% Survival 10 d post-hatch
Control ($n = 4$) vs. Cd diet ($n = 3$)	0.42	0.81	0.81	0.89	0.88
Irradiated ($n = 4$) vs. Cd diet and irradiated ($n = 4$)	0.11	0.12	0.19	0.27	0.84
Pooled non-irrad. ($n = 7$) vs. pooled irradiated ($n = 8$)	0.02	0.03	0.04	0.05	0.11

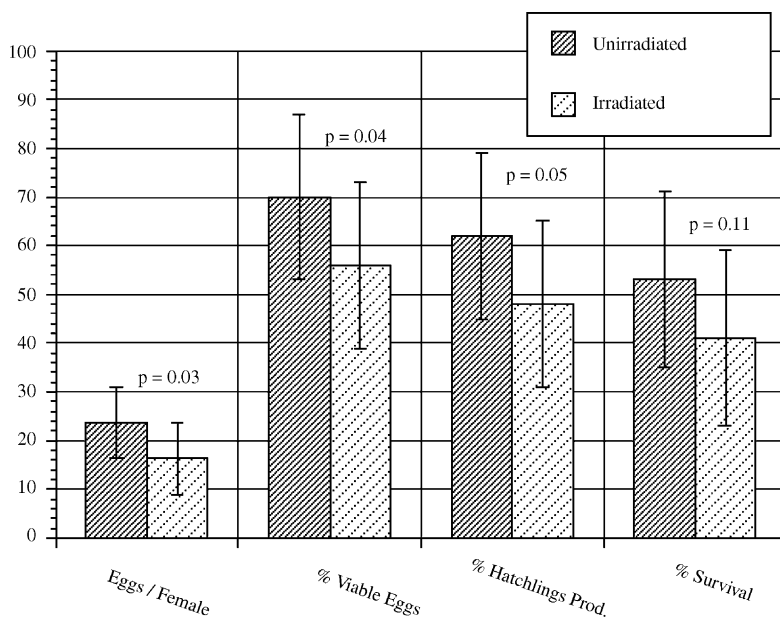


Fig. 4. Differences between irradiated ($n = 8$) and non-irradiated ($n = 7$) mesocosms for four population level endpoints measured in medaka fish. Endpoints included: (1) the mean number of eggs produced per female per day, (2) the percent of viable eggs produced, (3) the percent of hatchlings produced from the viable eggs, and (4) the percent survival of hatchlings measured 10 d post-hatching. Associated probabilities of results not being attributed to the irradiation treatments are shown for each endpoint, based on ANCOVA, with change in condition factor as a covariate.

4. Discussion

The reductions in these combined endpoints suggest that impacts to the fish population could occur from the chronic dose rates administered. However, a reduced fecundity at a dose rate of 350 mGy d⁻¹ is not unexpected. Reduced reproductive output in fish exposed to sub-lethal radiation levels were observed some 45 years ago (Donaldson and Foster, 1957). Declines in reproductive output of fish have been observed at dose rates lower than what we report herein. Complete sterility in *Ameioba splendens* was observed following 190 d of exposure to a ¹³⁷Cs source, at a dose rate of 185 mGy d⁻¹ (Rackham and Woodhead, 1984); and a 57 percent decrease in fecundity was seen in guppies (*Poecilia reticulata*) chronically exposed to 40 mGy d⁻¹ (Woodhead, 1977). Hyodo-Taguchi (1980) exposed male medaka to a ¹³⁷Cs source and then mated them to non-irradiated females. Sterility and the number of unfertilized eggs increased over controls at dose rates >65 mGy d⁻¹. *Tilapia mossambica* raised in ⁹⁰Sr-contaminated aquaria under chronic conditions with dose rates of 30–40 mGy d⁻¹ experienced total sterility (Voronina, 1973; as cited by Sazykina and Kryshev, 2003.) About 6% of the silver carp (*Hypophthalmichthys molitrix*) residing in the contaminated cooling pond of the Chernobyl nuclear reactor were sterile following exposure of <10 mGy d⁻¹ (Belova et al., 1993; as cited by Sazykina and Kryshev, 2003). Reduced fecundity in the roach (*Rutilus rutilus*) has been observed at dose rates >5 mGy d⁻¹ (Peshkov et al., 1978). Research on a freshwater fish similar in size to medaka, mosquito fish (*Gambusia affinis*), exposed to contaminated effluents from the Oak Ridge facility, revealed increased frequencies of dead and abnormal embryos at dose rates of 4 mGy d⁻¹ (Blaylock, 1969). Interestingly, the irradiated population studied by Blaylock (1969) had a significantly larger brood size that offset the increased mortality of embryos.

5. Conclusions and Future Directions

The reduced fecundity observed in this study are critical to future studies in the LoDIF. These results confirm the utility of our chosen model organism for detecting population-level responses from the highest dose rate possible in our facility. The results inform us of the level of variation expected in future research and thus sample sizes can be adjusted accordingly. Our challenge now is to conduct more extensive experiments at much reduced dose rates and to couple the observed population effects to other endpoints at lower levels of biological organizations (i.e. cellular and molecular effects). Currently our colleagues are working with medaka to:

- Develop methods to detect reciprocal translocations in chromosomes of medaka using fluorescence in situ hybridization with whole chromosome markers (laboratory of J. Bedford, Colorado State University, Fort Collins, CO). Reciprocal translations are biologically relevant because they can lead to a reduction in reproductive success through a process known as translocation heterozygosity, as has been demonstrated in a variety of organisms (Stern, 1973).

This type of chromosome aberration is also persistent in the body of exposed organisms and can be used as a biological dosimeter (Lucas et al., 1992).

- Develop micro-satellite assay methods for medaka (laboratory of T. Glenn, Savannah River Ecology Laboratory, Aiken, SC). Micro-satellite DNA loci are promising molecular genetic markers of environmental insults, and are increasingly used in genotoxicology studies (Brown et al., 2001)
- Develop transgenic strains of medaka that carry genes specifically designed to quantify mutations and to meet the need for rapid detection of tissue-specific mutations in a whole organism mutagen exposure (laboratory of R. Winn, University of Georgia, Athens, GA; Winn et al., 2000).
- Develop tiny TLD rods that can be placed in individual fish to obtain more accurate dose estimates (laboratory of J. Durham, Colorado State University, Fort Collins, CO).

Acknowledgements

This research was supported by the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, U. S. Department of Energy through Financial Assistant Award no. DE-FC09-96SR18546 to the University of Georgia Research Foundation. We are grateful to R. Winn and M. Norris for providing us with medaka and for valuable advice on fish husbandry, as well as Rahlé Marsh for assistance in the field.

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