- **Sublethal Effects of Tritium on Aquatic Systems**
- **Ecological Effects of Lithium and Beryllium on Important Aquatic Organisms and Associated Communities**
- o **Teratogenic Effects of Low-Level Magnetic Fields**

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Continuing studies of the sublethal effects of tritium on freshwater species emphasize the potential for genetic transmission of suppressed immune competence in offspring of parental rainbow trout (*Salmo gairdneri*) exposed to tritium (0, 0.04, 0.4, 40.0 rads) during embryogenesis. We plan to determine the relative biological effectiveness of tritium beta irradiation when compared to ^{60}Co gamma irradiation using the relatively radiosensitive immune process of rainbow trout. During FY 1979, we concluded genetic effects studies and prepared a manuscript for open literature publication summarizing FY 1977 and FY 1978 studies on the permanence of suppression of the primary immune response in rainbow trout sublethally irradiated during embryogenesis.

We are also studying the potential effects of beryllium and lithium on aquatic systems. Because of mining and refining subsequent to the use of these metals in the construction of fusion reactors, increased levels of each are likely to be encountered in surface waters. Studies included an evaluation of potential toxicity of lithium on embryological life stages of rainbow trout and an assessment of fate and effects in artificial stream habitats. Levels of lithium necessary to cause an observed effect would have to be at least three orders of magnitude above observed background.

Studies initiated in FY 1978 include evaluation of effects of low-level magnetic fields on embryologic development of rainbow trout. The objective of these studies to provide data useful in assessing potentially harmful effects of low-level magnetic fields encountered by attendant personnel working in the transport and hot cell areas of fusion reactors. This approach is less costly than using mammalian systems, provides large numbers of experimental organisms for meaningful statistical analysis and permits examination of potential latent effects in a representative vertebrate.

SUBLETHAL EFFECTS OF TRITIUM ON AQUATIC SYSTEMS

Genetic Effects Studies

Genetic effects studies first undertaken in FY 1978 were directed toward determining the potential for genetic transmission of suppressed immune competence to offspring from parental rainbow trout sublethally exposed to ^{3}HOH (0, 0.04, 0.4, 4.0, and 40.0 rads) over 21 days of embryogenesis. We had previously demonstrated that the primary immune response of parental fish was significantly suppressed and permanently altered at doses as low as 4.0 rads.

Offspring of each test cross (all test crosses were conducted within respective treatments) at five months of age were intraperitoneally injected with 0.1 ml of heat-killed antigen (1.8×10^{8} cells/ml Flexibacter columnaris in 25% Freund's incomplete adjuvant). A 0.1-ml sham vaccination (saline adjuvant) was similarly administered to a second group of control fish.

One week before vaccination, three weeks after vaccination, and at bimonthly intervals thereafter, a standard tube agglutination test for the specific antigen of vaccination was performed on the serum from 20 fish from each of the control and treatment test groups. Additional agglutination tests were performed when offspring were 12 months old.

Tritium-irradiated and control treatment groups were arranged among the available compartments of three concrete troughs according to a completely randomized block design (three blocks, five treatments). The data were treated by analysis of variance. All experimental groups sampled one week prior to vaccination failed to demonstrate positive agglutinins to F. columnaris at the lowest test dilution.

A clearly observable and statistically significant change in mean titer values occurred during the 11-week sampling period following vaccination (Figure 1). However, significant differences in mean titer values among the five groups of offspring at each

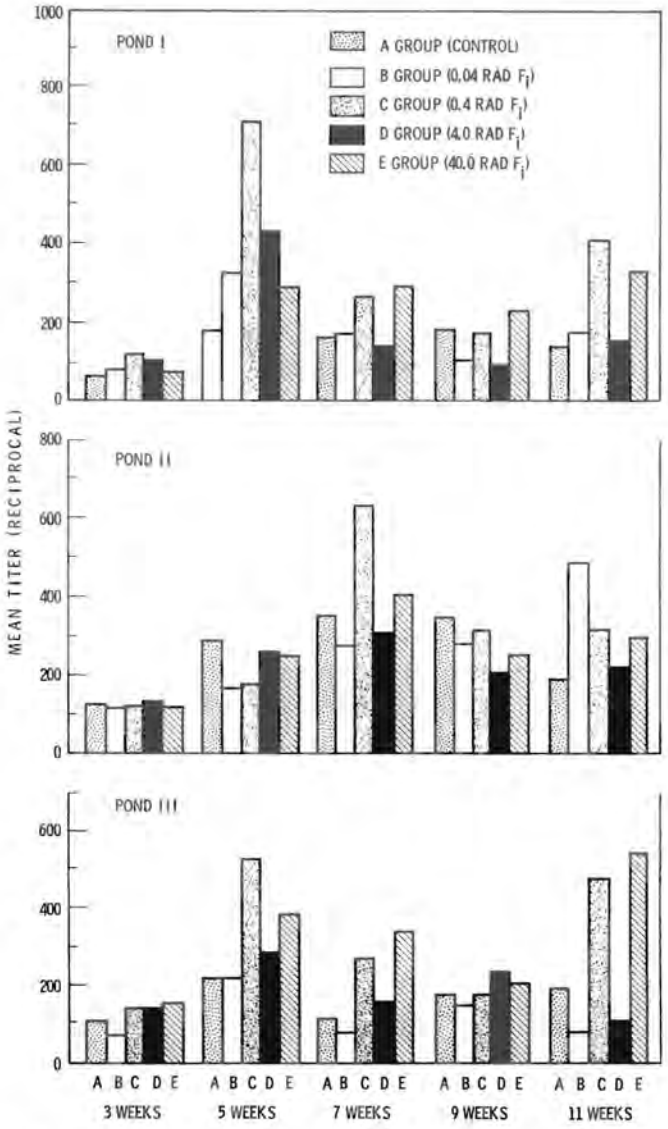

FIGURE 1. Mean Titer Values of Offspring for Specific Agglutinins to *Flexibacter columnaris* at Each Sampling Interval

sampling were not demonstrated. Serum agglutinins in sham-injected control fish were mostly nondetectable. Although a small percentage of the sham-injected fish demonstrated a positive titer, mean values of all positive titers were significantly lower than mean values of all positive titers in the control groups injected with antigen and adjuvant.

All experimental groups sampled 12 months after vaccination still demonstrated positive agglutinins to F. columnaris; but, as before, statistically significant differences in mean titer values among the five groups of offspring at each sampling were not detected. Accordingly, it could only be concluded that under the described experimental conditions suppressed immune competence as mediated by early radiation injury was not genetically transmitted.

Publications Activity

A manuscript for open literature publication summarizing studies on the permanence of immune suppression in rainbow trout following early radiation injury was prepared. Upon completion of internal review, it will be forwarded to Radiation Research.

Future Studies

In FY 1979 a proposal was prepared and forwarded to the Division of Health Effects Research with the objective of determining the relative biological effectiveness (RBE) of tritium beta irradiation when compared to ^{60}Co gamma irradiation applying the relatively radiosensitive immune process of the rainbow trout.

Much experimental work has been devoted to determining whether the high linear energy transfer of the tritium beta particle is reflected in an increased RBE when compared with gamma- or X-irradiation. The International Commission on Radiological Protection recognizes the RBE for tritium to be unity. More recently, however, there has been evidence to suggest a higher value is justified. Of particular note are the studies at Lawrence Livermore Laboratory on the survival of female germ cells in juvenile mice exposed to a continuously maintained level of tritium oxide in body water[a,b]. These studies would indicate an increasing RBE with longer exposure, with the suggestion of an RBE of 3 or 4 at very low doses (<20 rad). This question is of practical importance because tritium is, and probably will continue to be, a major contributor in the effluents from nuclear power plants, both fission and fusion designs. The radionuclide is not easily removed by conventional waste treatment practices. There is, therefore, increasing concern as to the ultimate concentration in, and the effect upon, biological systems. Furthermore, likelihood of tritium having increased biological effectiveness, particularly over extended low-level exposures, warrants careful examination.

ECOLOGICAL EFFECTS OF LITHIUM AND BERYLLIUM ON IMPORTANT AQUATIC ORGANISMS AND ASSOCIATED COMMUNITIES

The purpose of this study is to determine the ecological significance of elevated concentrations of lithium and beryllium in fresh water. The objective for this year was to identify parameters in freshwater biota that are perturbed by lithium and to relate the perturbations to the concentrations at which they were observed.

Observations to date indicate that lithium concentrations of ≥100 mg/ℓ reduce fertilization of rainbow trout (Salmo gairdneri) eggs by ≥50%, completion of embryogenesis by ≥95%, and hatching success by ≥95%. Lithium concentrations of ≥10 mg/ℓ caused ≥95% mortality in rainbow trout sac fry exposed during embryogenesis. Concentrations of ≥5 mg/ℓ caused 95% mortality in trout alevins after four days of exposure, and ≥3 mg/ℓ caused 95% mortality in rainbow trout fingerlings after the same exposure period. For juvenile rainbow trout, we observed that ≥2 mg/ℓ caused ≥90% mortality after 10 to 30 days of exposure.

In 14-day exposures of naturally occurring Columbia River periphyton communities to lithium, we observed that ≥25 mg of Li^+/ℓ reduced primary production by ≥95% and total biomass by ≥50%. In addition, the habitation of chironomid (midge) larvae living among the periphyton was reduced by ≥80% at lithium concentrations of ≥60 mg/ℓ.

A review of the literature indicates that natural background ranges of lithium in most freshwater systems occur from 0.0001 to 0.001 mg/ℓ, but may be as high as 0.1 mg/ℓ in lakes and streams with higher salinity, and sometimes exceeding 1.0 mg/ℓ in hot

(a) Dobson, R. L., J. H. Arrington and T. C. Kwan. 1975. Tritium Toxicity: Increased Relative Biological Effectiveness of ^3HOH with Protraction of Exposure. DOC. UCRL-76558.

(b) Dobson, R. L., and T. C. Kwan. 1976. "The RBE of Tritium Radiation Measured in Mouse Oocytes: Increase at Low Exposure Levels." Radiation Research 66:615-625.

springs and salt lakes. Thus far, investigations lead us to a tentative conclusion that lithium concentrations in most freshwater systems would have to be increased by at least three orders of magnitude to cause biological effects.

TERATOGENIC EFFECTS OF LOW-LEVEL MAGNETIC FIELDS

Influence of Magnetic Fields on Development of Rainbow Trout (Salmo gairdneri)

Previous studies suggested that fertilization, development and hatchability of rainbow trout eggs could be affected by exposure to magnetic fields. Additional studies were performed to confirm these results and to examine the relative susceptibility of sperm and egg to magnetic fields. Preliminary results and their interpretation are presented here for two different experimental approaches.

In the first approach, previously used, approximately 4,500 eggs were exposed from fertilization through 21 days in an oscillating field (2,800 to 11,200 gauss within a 1-min period) of a Varian Model V-3603 electromagnet. An additional 4,500 eggs maintained outside the magnetic field served as controls.

Incubation chambers for exposed and control embryos were identical. Subsampling of 150 embryos from both exposed and control chambers was conducted on days 7, 14, and 21. At 21 days (eyed stage), the embryos were transferred to standard drip incubators where hatching occurred in 28 $\pm$ 1.5 days. The fry were transferred to standard hatchery troughs two weeks after hatching to detect potential latent mortality and impaired growth. A chi-square test statistic was applied to each index of effect to determine differences between exposed and paired control groups. Evaluation of each index was conducted "in the blind" to decrease the possibility of investigator bias.

Table 1 presents results for three distinct stages of development, which include 1) fertilization, 2) hatching at 28 days, based on fertile embryos and 3) survival of hatched fry through 10 weeks. Fertilization success was higher in the exposed group than in the control group. No significant differences were detected for either percent hatchability or percent survival of hatched fry between exposed or control groups.

Results of subsampling are presented in Table 2. Combining all data for 7, 14 and 21 days indicated no significant difference in fertilization success between the exposed and control group, a result that contradicts enumeration of nonsubsampled embryos. Subsampling on day 7 revealed a higher incidence of abnormal embryos in the control group; however, this observation was not confirmed by subsampling at 14 and 21 days.

A second approach was designed to examine the fertilization process itself and to determine the relative susceptibility of egg and sperm to magnetic field effects. Accordingly, approximately 5,000 eggs and 50 ml of sperm were separately exposed in a homogeneous 11,200-gauss magnetic field for 1 hr. A similar number of eggs and aliquot of sperm were maintained outside the magnetic

TABLE 1. Effect of Magnetic Exposure on Early Life Stages of Rainbow Trout

Condition	Number of Eggs	Number Sub-sampled	Percent Fertile	x^2	Level	Percent Hatched	x^2	Level	Percent Survival at 10 Weeks	x^2	Level
Control	4511	754	85.23	—	—	99.31	—	—	99.40	—	—
Exposed	4388	749	89.42	29.28	(a)	99.48	0.72	(b)	99.72	3.76	(b)

(a)Significant x^2 at 0.05 level
(b)Nonsignificant x^2 at 0.05 level

TABLE 2. Effect of Magnetic Exposure on Incidence of Abnormal Embryos

Condition	Time, Days	Number Subsampled	Percent Fertile	x^2	Level	Percent Abnormal	x^2	Level
Control	7	149	93.29	—	—	10.79	—	—
Exposed	7	150	88.67	1.94	(b)	1.50	10.00	(a)
Control	14	155	87.74	—	—	0.74	—	—
Exposed	14	150	90.67	0.67	(b)	0.74	0	(b)
Control	21	150	84.00	—	—	0	—	—
Exposed	21	150	87.33	0.67	(b)	0.76	0.96	(b)

(a)Significant x^2 at 0.05 level
(b)Nonsignificant x^2 at 0.05 level

field. After exposure, eggs and sperm were placed in standard hatchery drip incubators in the following combinations: 1) egg unexposed, sperm unexposed; 2) egg exposed, sperm unexposed; 3) egg unexposed, sperm exposed and 4) egg exposed, sperm exposed. Subsampling was again performed on days 7, 14 and 21. Hatching occurred in 28 ± 1.5 days and fry were transferred to standard hatchery troughs two weeks after hatching.

Results (Table 3) suggested that there was a significant difference in fertility among the four treatment groups; however, again the lower percentage was encountered in the control group. While no significant difference in hatchability was found, these data suggested that survival through 10 weeks was significantly greater in the control group. A homogeneity X^2 test of all data ($p = 0.05$) supported these interpretations.

Results of statistical analyses to determine the relative influence of magnetic exposure on egg or sperm are shown in Table 4. Although these data suggested that magnetic exposure of either egg or sperm significantly influenced fertility and survival, the finding of a significant interaction diminished interpretation of main effects; that is, an additional influence on fertility and survival occurred that was not attributable to magnetic exposure of egg and sperm alone.

The findings reported in Table 5 suggested that a significant increase in the incidence

TABLE 3. Effect of Magnetic Exposure on Early Life Stages of Rainbow Trout

Condition	Number of Eggs	Number Subsampled	Percent Fertility	Percent Hatched	Percent Survival at 10 Weeks
Eggs/Sperm	2491	300	85.53	92.42	97.06
Eggs*/Sperm	2417	300	95.56	92.09	95.33
Eggs/Sperm*	2338	299	88.72	92.70	93.80
Eggs*/Sperm*	2509	300	95.29	92.16	95.77

*Gamete exposed to magnetic field

TABLE 4. Relative Influence of Magnetic Exposed Gamete on Fertility, Hatchability and Survival

	Fertility			Hatchability			Survival at 10 Weeks		
	d.f.	x^2	Level	d.f.	x^2	Level	d.f.	x^2	Level
Main Effect, Eggs*	1	187.78	(a)	1	1.82	(b)	1	0.01	(b)
Main Effect, Sperm*	1	7.57	(a)	1	0.09	(b)	1	7.52	(a)
Interaction, Eggs*/Sperm*	1	8.44	(a)	1	0.02	(b)	1	8.34	(a)

(a)Significant x^2 at 0.05 level
(b)Nonsignificant x^2 at 0.05 level
*Gamete exposed to magnetic field

TABLE 5. Effect of Magnetic Exposure on Incidence of Abnormal Embryos, Day 21

Condition	Normal Development	Abnormal Development
Eggs/Sperm	60	4
Eggs*/Sperm	70	4
Eggs/Sperm*	61	2
Eggs*/Sperm*	63	11

*Gamete exposed to magnetic field

TABLE 6. Relative Influence of Magnetic Exposed Gamete on Incidence of Abnormal Embryos, Day 21

	d.f.	x^2	Level
Main Effect, Eggs*	1	1.32	(b)
Main Effect, Sperm*	1	2.83	(b)
Interaction, Eggs*/Sperm*	1	4.10	(a)

(a)Significant x^2 at 0.05 level
(b)Nonsignificant x^2 at 0.05 level
*Gamete exposed to magnetic field

of abnormal embryos occurred in subsampling the group in which both egg and sperm were exposed to the magnetic field. A homogeneity X^2 test of all data ($p = 0.05$) supported this interpretation. Although not shown, analyses of subsamples collected on days 7 and 14 revealed no significant differences among the four treatment groups. As shown in Table 6, there was also no effect on development from exposing egg or sperm separately.