EFFECTS OF CHRONIC LOW-LEVEL IRRADIATION ON *GAMBUSIA AFFINIS*[1]

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IRRADIATED FISH POPULATION

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ABSTRACT

Since 1944, White Oak Lake (WOL), a 10.5-ha impoundment located on the U.S. Department of Energy's Oak Ridge Reservation, has served as a final settling basin for low-level radioactive effluents from the Oak Ridge National Laboratory. Organisms inhabiting the lake have been exposed for many generations to chronic low-level radiation significantly higher than background. During the past decade, studies on the mosquito fish, *Gambusia affinis*, from WOL have been carried out to relate estimated radiation doses to effects on the fitness of the *Gambusia* population. The purpose of this paper is to review the previous studies on *Gambusia* from WOL and to report on more recent results.

Results of studies on fecundity, temperature tolerance, and embryonic mortality have led to the conclusion that the *Gambusia* population in White Oak Lake has an increased frequency of deleterious and recessive lethal genes which may be attributed to the radiation exposure history. The frequency of nonviable embryos from WOL *Gambusia* did not change significantly from 1966 to 1978; however, it was still significantly greater than that of a control population. In July 1977, *Gambusia* from a control population were stocked into a 0.45-ha pond which had served as a low-level waste settling basin. The beta and gamma dose rate in this pond (measured at ten stations with thermoluminescent dosimeters) averaged from 37 rad/yr at the water surface, 394 rad/yr at mid-depth, and 1150 rad/yr at the surface of the sediments. Preliminary results from samples taken in August 1978

showed that although the frequency of nonviable embryos increased, the frequency was not significantly greater than that of the control parent population. Additional sampling of future generations of *Gambusia* in this pond will determine whether the frequency of nonviable embryos increases as succeeding generations are exposed to dose rates that are higher than the does rates in WOL.

INTRODUCTION

It is obvious that effects due to chronic low-level exposures are of greatest interest in considering possible ecological effects of man-made radiation, but surprisingly few studies have investigated aquatic populations exposed to continuous low-level radiation. Recent surveys of the literature (1,2) reveal that less than 10% of the papers on the effects of radiation on aquatic organisms refer to continuous or chronic irradiation.

White Oak Lake located on the U.S. Department of Energy's Oak Ridge Reservation is one of the few areas where such studies have been conducted. Aquatic organisms inhabiting this impoundment which serves as a final settling basin for radioactive effluents from the Oak Ridge National Laboratory (ORNL) are exposed to chronic low-level radiation significantly higher than background. Several studies have examined the effects of radiation on *Chironomus*, fish, and snail populations in White Oak Lake.

The frequency of chromosome aberations in *Chironomus* larvae was analyzed by Blaylock (3,4,5). Reproduction of the snail population, *Physa hetrostropha*, in White Oak Lake was compared to that of control populations (6). Fecundity and embryonic mortality in the mosquito fish, *Gambusia affinis*, from this lake was studied by Blaylock (7) and Trabalka and Allen (8). The purpose of this paper is to review the previous work on *Gambusia* in White Oak Lake and to report on more recent findings.

SITE DESCRIPTION AND RADIATION HISTORY

White Oak Lake was constructed in 1943 to serve as a final settling basin for radioactive effluents from the Oak Ridge National Laboratory (9). The impoundment, which had a surface area of about 15 ha, is located 1 km upstream from the confluence of White Oak Creek and the Clinch River. The temporary delay provided by the impoundment afforded some dilution and a period for the decay of short-lived radionuclides before release to the Clinch River. It also allowed the deposition and accumulation of contaminated sediment. In 1955 the lake was partially drained, leaving behind approximately 2.8×10^3 m^3 of contaminated sediment. The lake was then raised to about one-third of its previous capacity and at the present time has a surface area of about 10.5 ha.

The accumulated radioactivity in the sediments has been monitored at various times. Early estimates based on core samples ranged from 21 Ci in 1945 to 392 Ci in 1950. A later detailed study of the radionuclides in White Oak Lake sediments in 1962 (10) estimated that ^{106}Ru (1038 Ci) and ^{137}Cs (704 Ci) accounted for 90% of the activity in White Oak Lake. Cobalt-60 (152 Ci), ^{90}Sr (15 Ci), and trivalent rare earths exclusive of ^{90}Y made up the remainder of the activity in the sediments. More than half of the activity in the sediments was found in the 0 to 15-cm layer of sediment.

The release of radioactive effluents to White Oak Lake has decreased significantly since a high of 2196 Ci in 1960 (Table I). The greatest amount of activity in the sediments is now located 15 cm or more below the surface of the sediment.

Table I. Annual discharge (in curies) of radionuclides from White Oak Lake to the Clinch River

Year	^{137}Cs	^{106}Ru	^{90}Sr	^{60}Co	Others	Total[a]
1959	76	520	60	77	200	933.0
1960	31	1900	28	72	165	2196.0
1961	15	2000	22	31	124	2192.0
1962	5.6	1400	9.4	14	24	1453.0
1963	3.5	430	7.8	14	13	468.3
1964	6.0	191	6.6	15	15	234.6
1965	2.1	69	3.4	12	7	93.5
1970	2.0	1.2	3.9	1	6	14.1
1975	1.2	0.2	6.0	0.6	5	13.0

[a]Excludes ^{3}H.

DOSE RATES TO AQUATIC ORGANISMS

One of the major difficulties in studying the effects of radiation on aquatic organisms in White Oak Lake is that of assigning a dose to a particular organism or a dose rate to a population. The uneven distribution of radionuclides in bottom sediments and mobility of organisms, especially fish, complicate any dose calculation or measurement.

After the lake was drained in 1955, Lee and Auerbach (9) used ionization chambers and film dosimeters to measure the radiation field one meter above the lake bed. The lake was divided into areas depending upon dose rates. Near the shore of the former lake, the dose rate ranged from 0 to 10 mr/hr, while at the upper end of the former lake bed the dose ranged from 40 to 50 mr/hr. A maximum dose rate of 100 mr/hr was measured near the inlet of White Oak Creek. In 1961, maximum dose rates of 2 rad/hr were measured above the east stream which enters the upper end of White Oak Lake (11). At this time the east stream was receiving seepage from a ^{106}Ru waste disposal pit. Nelson and Blaylock (12) in 1961 calculated that Chironomus larvae living in the sediment directly below White Oak Dam received a dose of 230 rad/yr or 1000 times background. Larvae living directly in the radioactive sediment received a much higher dose than fish swimming above the sediment where they were partially shielded by the overlying water.

In 1965 the dose rate to Gambusia swimming 10 cm above the surface of the sediment was calculated as 10.9 rad/day (7); however, these calculations were showed to be in error (8). In 1969 Cooley (6) calculated a beta and gamma dose rate of 0.65 rad/day to snails living in the east stream. A significant contribution to the dose rate was made from the concentration of radionuclides in the algae. In 1971 Toshiba metaphosphate glass dosimeters were used to measure a dose rate of 0.64 rad/day at the surface of the sediments in the upper end of the lake.[2] Trabalka and Allen (8) exposed thermoluminescent dosimeters for 14 days on the mud flats in the upper end of White Oak Lake in June 1975. The average dose rate 11 cm above the sediment surface was 0.06 rad/day, while on the sediment surface the average was 0.12 rad/day.

These measurements and dose calculations show a significant decrease in the dose rate to aquatic biota in White Oak Lake during the last decade. They also emphasize the difficulty of determining a dose rate for a specific organisms or population. Undoubtedly the Gambusia population located in the upper end of White Oak Lake has received several thousand rads of irradiation since 1945.

MATERIAL AND METHODS

The mosquito fish, *Gambusia affinis* (Baird and Girard), is viviparous, with several broods being produced during a breeding season. In each female, ova mature and are fertilized at approximately the same time. The gestation period is 21 to 28 days. After the birth of a brood, other ova mature and are fertilized to produce the next brood.

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[2]E. B. Douple, Unpublished Data, Oak Ridge National Laboratory, Oak Ridge, Tennessee (1971).

Similar techniques were used in the various studies in White Oak Lake to determine the effects of radiation on the <u>Gambusia</u> populations. Gravid females with well-developed embryos were dissected to determine the number of viable, nonviable, and abnormal embryos. The abnormal embryos were viable embryos having malformations, the most obvious being twinning and vertebral malformations.

<u>GAMBUSIA</u> STUDIES IN WHITE OAK LAKE

The first study on <u>Gambusia</u> in White Oak Lake was initiated in 1965 (7) to examine the possible effects that exposure to chronic low-level radiation for many generations would have on the <u>Gambusia</u> population. The brood size, which was considered synonymous with fecundity, was determined in <u>Gambusia</u> from White Oak Lake along with viable and nonviable embryos. The frequency of these parameters in White Oak Lake <u>Gambusia</u> was compared with those of control population. Since it was known that the length of the female and the time sequence of the broods influenced the fecundity, a statistical analysis of the brood size considering these factors was developed. A significant difference in fecundity was found between the White Oak Lake population and the control population. Surprisingly the irradiated population contained a significantly greater number of viable embryos than did the control populations. A follow-up study (13) which included two additional control populations gave similar results.

Although fecundity of <u>Gambusia</u> from White Oak Lake was significantly greater than that of the control population, the frequency of dead and abnormal embryos was also significantly greater than that of the controls. The increased frequency of nonviable embryos led to the conclusion that the irradiated White Oak Lake population contained a higher frequency of deleterious and recessive lethal genes in its gene pool which could be attributed to its radiation exposure history.

The increase in fecundity in Gambusia from White Oak Lake was more difficult to explain. It was known that the fecundity of Gambusia may vary from locality to locality. Although the precise reason for this variation is not known, the fertility of the body of water is suspect. Another reason for the increased fecundity could be the exposure to chronic irradiation. Several papers (14,15,16) have reported increased fecundity following exposure to ionizing radiation. For this and other reasons it was hypothesized (in 1966) that the increased fecundity of the females in White Oak Lake population may be an adjustment to the exposure to chronic irradiation which produced an increased frequency of nonviable embryos.

In 1973, based on results obtained in laboratory and field experiments, Trabalka and Allen (8) concluded that the increased fecundity in Gambusia from White Oak Lake was probably a function of the high productivity in this eutrophic environment. A comparison of the 1973 data with the 1966 results showed that the fecundity and frequency of nonviable embryos in Gambusia from White Oak Lake had not changed and were still significantly greater than that of control populations (Table II).

Table II. Viability of *Gambusia* embryos from the White Oak Lake (WOL) field population and from laboratory-reared White Oak Lake and control populations[a]

Year and population	Number of females	Total number of embryos	Percentage nonviable embryos
1973 - WOL field	30	2061	5.97
1974 - WOL laboratory	25	605	5.45
1974 - Pooled lab control	101	3614	2.79

[a]from Trabalka and Allen (8).

Trabalka and Allen (8) extended their work to the laboratory to remove the influence of variable environmental factors. Laboratory-born progeny of <u>Gambusia</u> from White Oak Lake and four control populations were maintained under similar laboratory conditions until they were ready to reproduce. Although the fecundity of each population was reduced, there was no significant difference in fecundity among the populations. Whereas the fecundity of White Oak Lake <u>Gambusia</u> had been significantly greater than the control population, after one year in the laboratory there was no significant difference in the fecundities. Conversely, the frequency of nonviable embryos in the laboratory population from White Oak Lake had not changed significantly; the frequency was still significantly greater than that of the control populations. These data support the conclusion that the increased frequency of nonviable embryos in <u>Gambusia</u> from White Oak Lake results from an increase of deleterious and recessive lethal genes in the gene pool. However, Trabalka and Allen's (8) results appear to refute the hypothesis that the increased fecundity in White Oak Lake <u>Gambusia</u> is genetically controlled; nevertheless, there is one questionable aspect of their data: each of their laboratory populations suffered a significant decrease in fecundity; however, this is often characteristic of laboratory populations which are maintained under conditions (i.e., temperature, light, diet, etc.) that are different from natural populations.

FITNESS OF *GAMBUSIA* FROM WHITE OAK LAKE

Generally it is assumed that an increase in embryonic mortality would have a detrimental effect on the fitness of a population. However, it would be difficult to conclude that such a population would have a lower fitness, if that same population produced a greater number of viable embryos; that is, providing fitness is accepted as a multidimensional entity which represents the average ability of a genotype to pass its genes to the next generation.

In another experiment Trabalka and Allen (8) used a Critical Thermal Maxima Test (17,18) to measure the difference between *Gambusia* from White Oak Lake and control populations. The critical thermal maximum temperature is defined as the arithmetic means of the collective thermal points at which locomotory activity becomes disorganized and an animal loses its ability to escape. Both the mean and variance of the critical thermal maximum temperature of White Oak Lake male *Gambusia* from the laboratory population were significantly different from the control populations. These results also support the previous conclusion that the *Gambusia* population in White Oak Lake has an increased genetic load (increased frequency of deleterious and recessive lethal genes).

Although the data are convincing that the White Oak Lake population has a higher concealed genetic load, the fitness of the population may not be reduced. According to *Drosophila* investigators, the magnitude of the genetic load is not an index of fitness (19).

Data collected in 1978, showed that the frequency of nonviable embryos has not changed significantly ($X^2 = 0.96$, d.f. = 3, $P > 0.25$) from 1966 to 1978 (Table III). Although, the radiation dose rate to the Gambusia in White Oak Lake has continued to decrease for the past decade, the frequency of nonviable embryos has remained relative constant. These results are consistent with findings from Drosophila populations where an elevated level of deleterious and recessive lethal genes was maintained in the population after the exposure to radiation had ceased (19).

OTHER FACTORS WHICH COULD INCREASE NONVIABLE EMBRYOS

White Oak Lake receives chemical pollutants in waste effluents which could possibly produce nonviable embryos in the Gambusia population. In the past, chromates from cooling tower blowdown have entered the lake. Elwood et al. (20) found that the level of Cr in bluegill (Lepomis macrochirus) and largemouth bass (Micropterus salmoides) from White Oak Lake was not significantly different from that of a control population from a lake not contaminated with Cr. The level (0.59 ppm) of Cr in Gambusia from White Oak Lake was not significantly different from the bluegill or largemouth bass; however, the effect of this level of Cr on the viability of Gambusia embryos is unknown.

Gambusia in White Oak Lake are often heavily infested with parasites. In 1969, Holland (21) investigated the possible influence of metacercariae belonging to the genus Posthodiplostomum on the reproductive potential of Gambusia. In this detailed study on Gambusia

Table III. The percentage of nonviable embryos in _Gambusia affinis_ from White Oak Lake from 1966-1978

	Year			
	1966	1967	1973	1978
Total number of embryos	4625	3745	2061	563
Percentage of nonviable embryos	5.92	5.93	5.97	4.97

from White Oak Lake, no relationship was established between heavily parasitized fish and fecundity or embryonic mortality.

NONVIABLE EMBRYOS IN GAMBUSIA AT HIGHER DOSE RATES

A study to examine the effects of radiation at higher dose rates than those that occur in White Oak lake was initiated in 1977 in a 0.45-ha decommissioned waste pond at ORNL. This pond had received liquid effluents from a low-level waste plant since 1944. An inventory of the radioactivity in the pond in 1977 indicated that the sediments contained approximately 5.0 Ci of $^{239,240}Pu$, 200 Ci of ^{137}Cs, and 33 Ci of ^{90}Sr. After the releases from the waste plant ceased, the pond developed native flora and fauna and is now being used to investigate the cycling of transuranics in a freshwater pond.

The dose rate in the pond was measured at ten locations near shore by exposing thermoluminescent dosimeters for a period of two weeks. The beta and gamma dose rate in the pond averaged 37 rad/yr at the surface of the water, 394 rad/yr at mid-depth, and 1150 rad/yr at the surface of the sediments.

In July 1977, Gambusia from a nonirradiated control population was stocked in the pond. The stocking was successful and in July and August of 1978, after approximately three generations, gravid females were collected to determine the frequency of viable and nonviable embryos (Table IV). Although the frequency of nonviable embryos in the pond population had increased from 2.60 to 4.10%, the difference was not significant (X^2 = 2.05, d.f. = 1, P > 0.10). The fecundity data for these preliminary samples were not analyzed because of the

Table IV. Percentage of nonviable *Gambusia* embryos in a control population and in the descendents of the same population after one year or approximately three generations of exposure to chronic irradiation in a radioactive waste pond

Year and population	Number of females	Total number of embryos	Percentage nonviable embryos
1977, control parent	17	954	2.60
1978, waste pond	15	390	4.10

small sample size and the time over which they were collected. Additional sampling of the population after more generations have been exposed will determine whether the frequency of nonviable embryos has increased significantly.

SUMMARY

The dose rate to *Gambusia* in White Oak Lake has decreased as the radioactive effluents entering the lake have decreased from a high of 2195 Ci in 1960 to 13 Ci in 1975. The frequency of nonviable embryos was significantly greater in the population of *Gambusia* in White Oak Lake from 1966 to 1978 and in the progeny of laboratory-reared females from White Oak Lake than in control populations. *Gambusia* from White Oak Lake had a significantly lower mean temperature and showed more variability in their response to a Critical Thermal Maxima Test than control populations. These results have led to the conclusion that the *Gambusia* population in White Oak Lake has an increased frequency of deleterious and recessive lethal genes in its gene pool. This increased genetic load has been attributed to past radiation history; however, chemical pollutants which could be mutagenic have also been released to White Oak Lake.

The increased frequency of nonviable embryos and the lower thermal maximum temperature in *Gambusia* from White Oak Lake could be considered detrimental to the population. However, it is difficult to assign a lower fitness to the population because the females produce a significantly greater number of viable embryos.

An experiment was initiated in 1977 by stocking Gambusia from a control population in a decommissioned waste pond where the dose rate at the sediment surface averaged 1149.6 rad/yr. Preliminary results from this experiment showed an increased frequency of nonviable embryos; however, the increase was not significantly greater than that of the control parent population. Additional sampling as more generations are exposed to irradiation will determine whether the frequency of nonviable embryos will become significantly different from the control parent population.

Despite the difficulties encountered in studying natural populations exposed to chronic irradiation, such studies provide insight into the effects of radiation which cannot be gained in laboratory experiments. Few areas exist where long-term studies can be conducted on populations exposed to chronic low-level irradiation significantly higher than background radiation. Therefore, every reasonable opportunity should be taken to investigate such situations.

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