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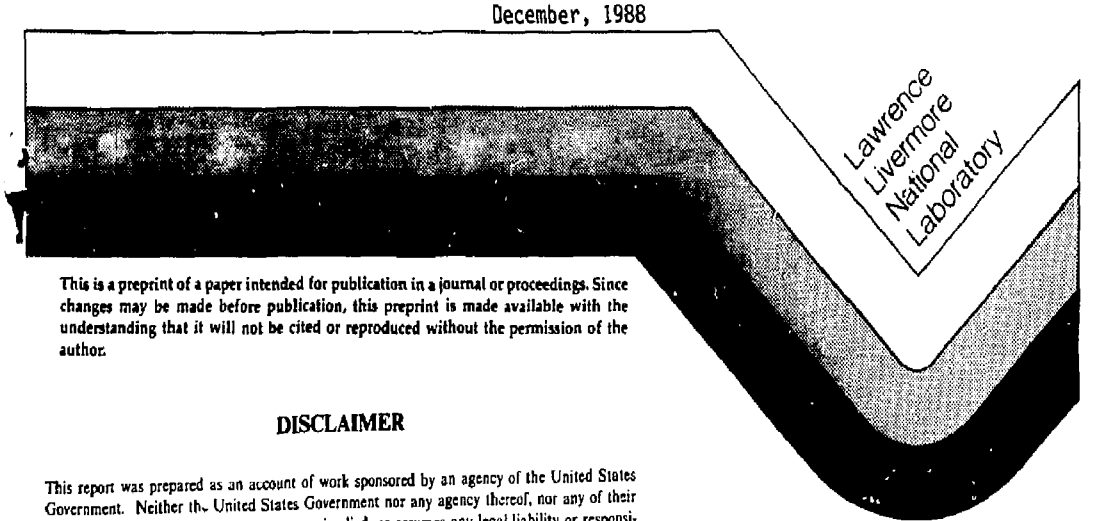

THE EFFECTS OF CHRONIC RADIATION ON REPRODUCTIVE SUCCESS OF THE POLYCHAETE WORM NEANTHES ARENACEODENTATA

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FOREWORD

In response to the mandate of Public Law 92-532, The Marine Protection, Research, and Sanctuaries Act of 1972, as amended, the Environmental Protection Agency (EPA) has developed a program to promulgate regulations and criteria to control the ocean disposal of low-level radioactive wastes. The EPA seeks to understand the mechanisms for biological response of marine organisms to the low levels of radioactivity that may arise from the release of these wastes as a result of ocean disposal practices. Such information will play an important role in determining the adequacy of environmental assessments provided to the EPA in support of any disposal permit applications. Although the EPA requires packaging of low-level radioactive wastes to prevent release during radiodecay of the materials, some release of radioactive material into the deep-sea environment may occur if a package deteriorates. Therefore, methods for evaluating the impact on biota are being evaluated.

Mortality and phenotypic responses are not anticipated at the expected low environmental levels that might occur if radioactive materials were released from the low-level waste packages. Therefore, traditional bioassay systems are unsuitable for assessing sublethal effects on biota in the marine environment. The EPA Office of Radiation Programs has had an ongoing program to examine sublethal responses at the cellular level, using cytogenetic endpoints.

The present study examines the effects of chronic radiation on the reproductive success of the marine polychaete, Neanthes arenaceodentata, a low-fecund invertebrate species. Data were generated through the second filial generation on brood size, abnormal development, and numbers of embryos living, dying, and dead following lifetime exposure to radiation.

The results of this research may be useful in evaluating ocean disposal of other materials because many other pollutants are also mutagenic. Cellular level endpoints and those indicative of reproductive success, and therefore predictive of population-level impacts, could ultimately be used to compare the risks of several pollutant classes.

The Agency invites all readers of this report to send any comments or suggestions to David E. Janes, Director, Analysis and Support Division, Office of Radiation Programs (ANR-461), U.S. Environmental Protection Agency, Washington, DC 20460.

Richard J. Guimond, Director
Office of Radiation Programs (ANR-458)

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ABSTRACT

The effects of lifetime exposure to chronic irradiation on reproductive success were assessed for laboratory populations of the polychaete worm Neanthes arenaceodentata. Lifetime exposure was initiated upon the spawning of the P_1 female and was terminated upon spawning of the F_1 female. Groups of experimental worms received either no radiation (controls) or 0.19, 2.1, or 17 mGy/h. The total dose received by the worms was either background or approximately 0.55, 6.5, or 54 Gy, respectively. The broods from the F_1 mated pairs were sacrificed before hatching occurred, and information was obtained on brood size, on the number of normal and abnormal embryos, and on the number of embryos that were living, dying, and dead.

The mean number of embryos in the broods from the F_1 females exposed to lifetime radiation of 0.19 and 2.1 mGy/h was not significantly different from the mean number of embryos from control females; however, the mean number of embryos was different from those F_1 females exposed to 17 mGy/h. There was a significant reduction in the number of live embryos in the broods from the F_1 mated pairs that were exposed to the lowest dose rate given, 0.19 mGy/h, as well as those exposed to 2.1 and 17 mGy/h. Also, increased percentages of abnormal embryos were determined in the broods of all the radiation-exposed groups.

Our results on embryo abnormalities and mortalities indicate that dominant-lethal mutations, and possibly recessive-lethal mutations, were most likely induced in the germ cells and that these mutations had an adverse effect on reproductive success by affecting the survival of early life stages. Except for those mated pairs exposed to 17 mGy/h, there was no evidence of gamete killing, nor was there evidence of reduced fertilization success because the number of developing embryos in the broods did not decrease with increased dose. From our data on estimated hatch size and actual hatch size, we concluded that doses as low as 0.19 mGy/h can reduce significantly the size of hatches when lifetime doses are given.

1. INTRODUCTION

One of the problems facing managers and scientists concerned with the impact of contaminants on aquatic environments is assessment of the effects of chronic exposure to sublethal levels of potentially toxic materials. One special concern is the response of aquatic organisms to long-term exposure to direct- and indirect-acting mutagens; exposure to mutagens can result in alterations in genetic material in both somatic and germ cells (UNSCEAR, 1986). Important detrimental effects of mutagens in somatic cells are the induction of tumors and cancer. Important detrimental effects on germ cells are the induction of dominant- and recessive-lethal mutations, cell killing, and the development of abnormalities in early life-history stages, all of which are factors that affect reproductive success. Because preservation of the health of aquatic environments requires insuring the maintenance of indigenous populations as well as the survival of individuals, managers of aquatic resources are concerned about the impacts of contaminants on reproductive success.

A direct-acting mutagen for which there is considerable data is ionizing radiation (NRC 1980; UNSCEAR 1977, 1982, 1986). Ionizing radiation is a genotoxic agent for which the dose to aquatic animals can be determined accurately without parallel studies on chemical metabolism. Ionizing radiation is an ideal model mutagen because the nature of the damage and the processes that modify the lesions are well characterized. Data on the effects of radiation on aquatic organisms have been reviewed extensively (Polikarpov 1966; Templeton et al. 1971; Templeton 1976; Chipman 1972; Ophel 1976; Blaylock and Trabalka 1978; Egami and Ijiri 1979; Woodhead 1984; Anderson and Harrison 1986). However, the great preponderance of the data is on acute rather than chronic effects.

The extensive data on the effects of acute radiation on mortality rates in aquatic animals appear to indicate that the radiosensitivity increases with biological complexity, i.e., that the higher the phylogenetic position, the lower the LD_{50} (Templeton 1976; Blaylock and Trabalka 1978; Woodhead 1984). However, the limited data on effects of acute radiation at the cellular level indicate that this conclusion may not be valid. Induction of chromosomal aberrations and sister chromatid exchanges by acute radiation in the polychaete Neanthes arenaceodentata occurred at doses that did not differ greatly from doses inducing such responses in some mammals (Harrison et al. 1986; Anderson et al. 1987). Furthermore, some fishes and invertebrates are as sensitive to radiation as some mammals (Rackham and Woodhead 1984; Harrison and Anderson 1988; UNSCEAR 1986), although the data on the effects of radiation on reproductive success indicate that there is considerable variation among species (see reviews of Woodhead 1984; Anderson and Harrison 1986).

The impact of radiation on the reproductive success of an aquatic organism may be related not only to the sensitivity of its gametes but also to its reproductive strategy. In a highly fecund species, the survival of early life stages may be less than 1%, and the loss of abnormal embryos induced from radiation exposure may be masked completely by those lost from density-dependent factors, such as food limitation and predation. It might be expected that the impact of radiation exposure to a species of low fecundity may be considerable because recruitment is more closely related to parent stock size. The limited data available on the use of sealed sources for the

chronic exposure of fish are not sufficient to allow conclusions to be drawn. Woodhead (1977) found reduced fecundity in the guppy (a low-fecundity species, from a lifetime exposure to about 1.7 mGy/h, while Welander et al. (1948) noted some long-term deleterious effects in salmon (a high-fecundity species) at about 2.1 mGy/h.

The objective of this study was to obtain information on the effects of chronic radiation on the reproductive success of a relatively low-fecundity invertebrate marine animal. The species selected was Neanthes arenaceodentata, which is a polychaete worm that is available commercially, is easily maintained in the laboratory, and for which considerable information is available on effects from acute radiation (Harrison et al. 1986; Anderson et al. 1987; Harrison and Anderson 1988) and from toxic inorganic and organic contaminants (Rossi and Anderson 1978; Oshida et al. 1981; Oshida and Ward, 1982). The data obtained from this study on effects of chronic exposure to the direct-acting mutagen, radiation, should be useful in evaluating ocean disposal of radioactive materials as well as other mutagens. Also, comparison of data for worms exposed chronically to data for worms exposed acutely will provide information on the importance of total dose and dose rate on response to radiation.

2. MATERIALS AND METHODS

2.1 Experimental Approach

The effect of chronic lifetime radiation on the reproductive success of N. arenaceodentata was determined by making observations on control and radiation-exposed worms. Data were obtained on the parental (P_1), first filial (F_1), and second filial (F_2) generations. Lifetime exposure to radiation was initiated upon the spawning of the P_1 female. At that time, these embryos, which were being cared for by the male, were placed in front of a radiation source. The lifetime exposure was terminated upon the spawning of the F_1 female (Fig. 1). The number of gravid females (P_1) used as sources for embryos for the control group was 6, for those receiving 0.19 and 2.1 mGy/h was 7, and for those receiving 17 mGy/h was 3. The total number of broods analyzed for the control group was 94, for the group receiving 0.19 mGy/h was 84, for the group receiving 2.1 mGy/h was 80, and for the group receiving 17 mGy/h was 59. Numbers of offspring of the P_1 and F_1 generations were determined as well as the times of spawning, hatching, and exiting of larvae from the parental tube. In addition, for both control and radiation-exposed F_1 mated pairs, the embryos in the brood were examined for abnormalities and subjected to a dye-exclusion test to determine the number that were living, dying, and dead. Data accumulated on the brood from each F_1 mated pair are provided in the Appendix.

Animal Sources, Culture Conditions, and Irradiation

Worms used in the experiment were obtained either from Dr. Donald Reish (California State University, Long Beach, CA) or from Brezina and Associates (Dillon Beach, CA). After the adult worms were received from the suppliers, they were held in 80-L aquaria for several weeks. Once the female worms began to develop oocytes, they were removed from the aquaria, mated with vigorous males from the same supplier, and cultured according to procedures by Reish (1974). Oocytes in the coelom of N. arenaceodentata are clearly discernable because the cuticle is translucent. Each mated pair (P_1) was placed in a

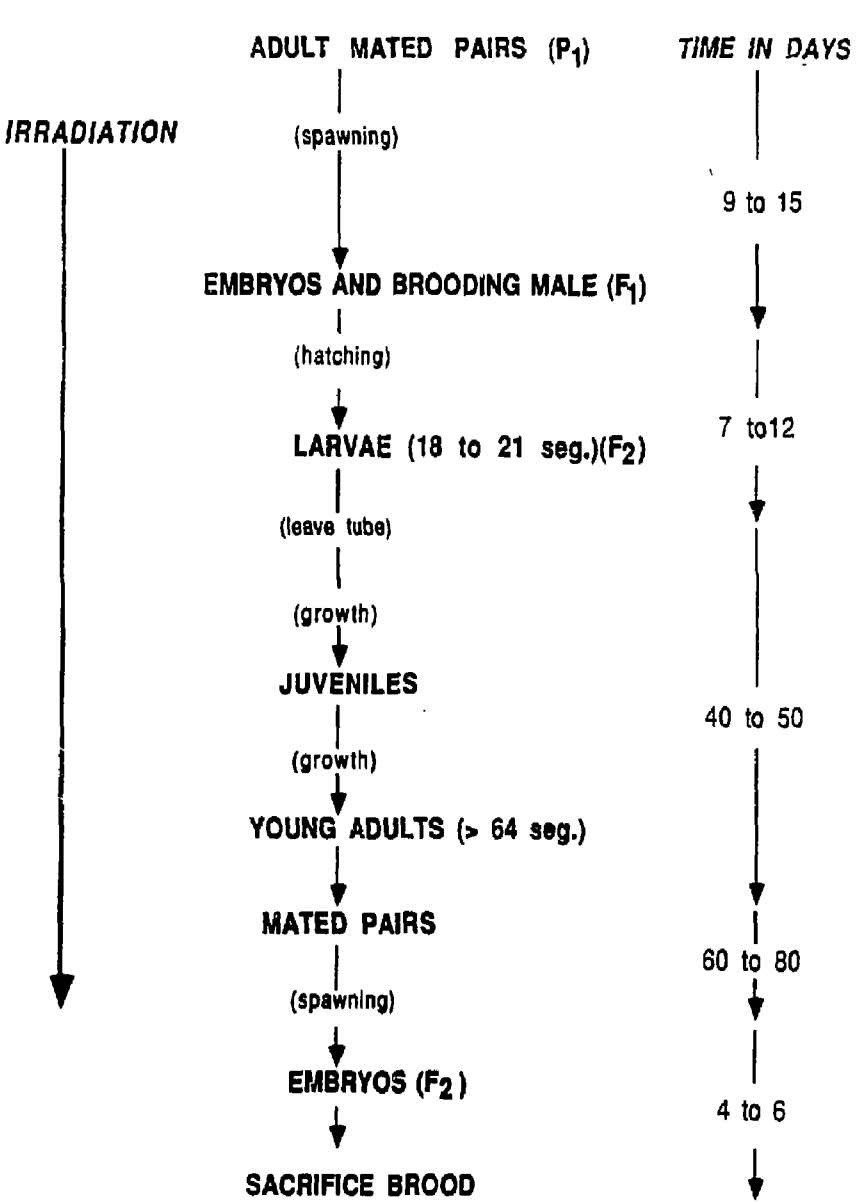

Figure 1. Summary of the life-history stages and of the steps in the procedure followed to determine the effects of radiation on reproductive success of Neanthes arenaceodentata.

large plastic petri dish (120-mm diameter x 20-mm depth) containing about 80 mL of filtered (1.0-μm pore size) seawater; tube formation occurred within the next 24 h. Seawater used in the experiments was pumped from the Pacific Ocean and passed through sand filters at the University of California Bodega Marine Laboratory before it was transported to the Lawrence Livermore National Laboratory; the seawater was stored before use in an underground 40,000-L tank.

During the acclimation period, observations of the mated pairs were made twice weekly. At these times, most of the seawater in the dishes was decanted, the tubes were carefully trimmed, excess mucus and fecal material were removed by wiping out the dish except in the tube area, newly filtered seawater was added, and fresh food was supplied (rehydrated freeze-dried Enteromorpha sp.). When the female stopped eating, which occurred when her coelom was filled with oocytes, the mated pair was transferred to the control area of the radiation facility and was observed daily to determine the day of spawning.

Irradiation of the embryos was initiated immediately after spawning occurred. The date of spawning was recorded, the female, who dies after spawning, was removed from the petri dish (if she had not been eaten by the male), and the petri dish containing the brooding male and the embryos was placed randomly in standard commercial petri-dish racks that held 18 petri dishes (two stacks each of 9 petri dishes). The radiation delivered was from a ^{60}Co source (about 2.5×10^{10} Bq; 0.7 Ci). The racks were located in one of following four areas in the radiation facility: behind the radiation source in a lead-shielded site (control area) or at one of three sites increasingly distant from the radiation source (irradiation areas) (Fig. 2). The three distances from the source were chosen in advance so that the worms in the petri dishes would be dosed at a rate of either approximately 0.21, 2.1, or 21 mGy/h (about 0.5, 5.0, or 50 rad/d). However, actual dose rates delivered were 0.19 ± 0.03, 2.1 ± 0.4, and 17 ± 1.1 mGy/h. Because the area in front of the source from which a dose rate of 17 mGy/h could be delivered was limited, the number of broods exposed at this dose rate was smaller than those at the two lower dose rates. The temperature in the exposure facility was $20 \pm 2°C$, and the light level was low during the day, except during the maintenance periods.

Doses delivered to the worms were monitored using thermoluminescent dosimeters. These were sealed in plastic and placed in the seawater in the petri dishes at positions similar to those occupied by the worms. Sets of dosimeters were used at each of the three distances from the source and were added at different times during the experiment. From the knowledge of the radiation exposure obtained from the dosimeters, of the number of days each worm was exposed to the source, and of the total time the radiation source was down during maintenance and feeding of the worms, the total lifetime dose received by each worm was calculated.

The broods were observed twice weekly, and care was taken to minimize any disturbance of the brood; the seawater was not changed unless it appeared to be becoming stagnant. The amount of food given was reduced and was placed at the opening of the tube. The date of hatching of the larvae, which occurred generally between 12 and 15 d after the spawning, was noted as well as the date that the larvae left the tube, which occurred between 7 and 12 d after the time of hatching.

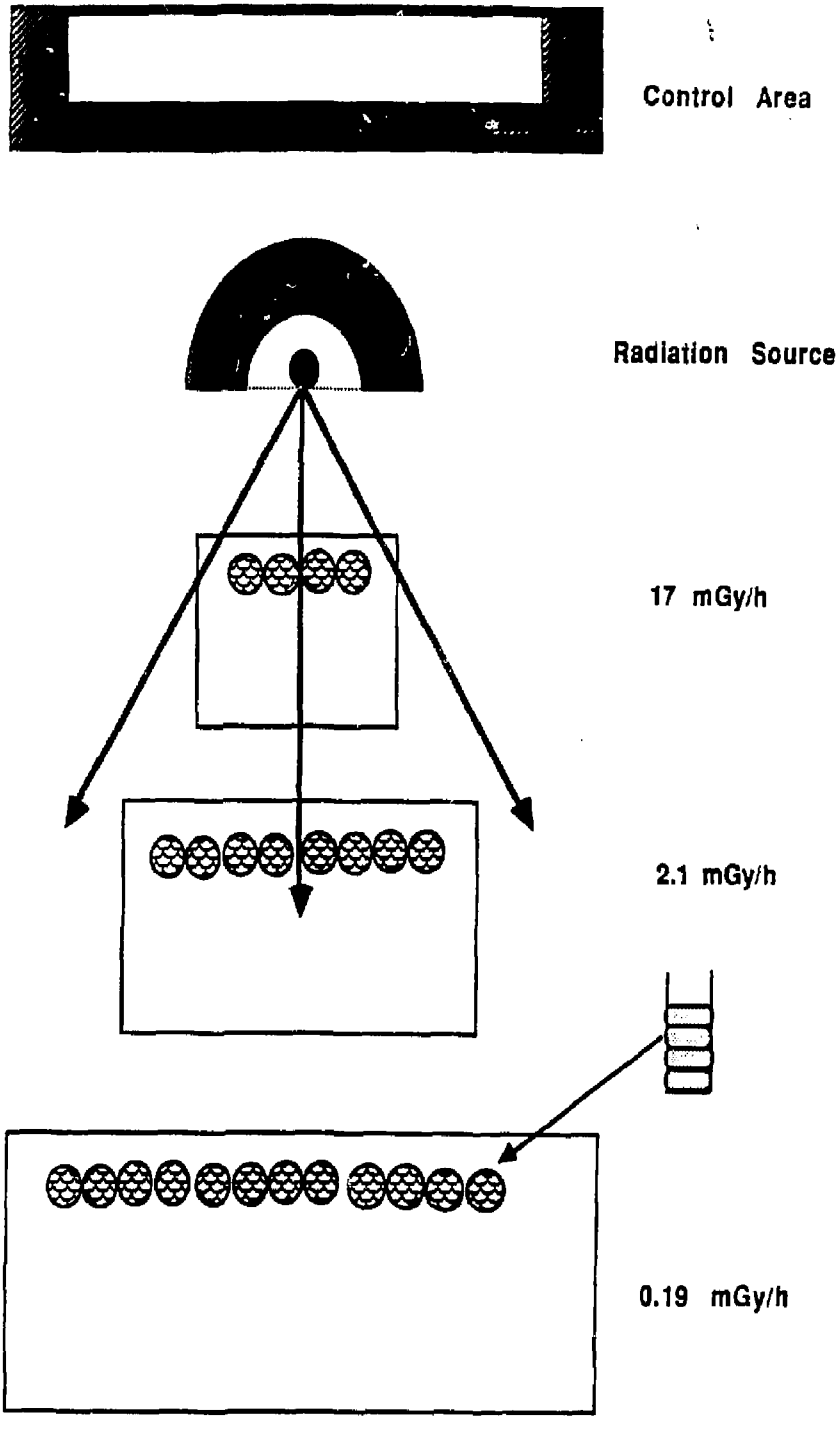

Figure 2. Schematic diagram of the radiation-exposure facility. The ^{60}Co source and the control zone were shielded heavily with lead.

When most of the larvae in the brood left the tube (larvae had from 40 to 70 segments), the larvae were removed from the large petri dish and three larvae, each from the same brood, were placed in a small petri dish (60-mm diameter x 20-mm depth) containing 10 to 15 mL of filtered seawater. Each small petri dish, containing three larvae, was placed in a standard commercial petri-dish rack in the same experimental area as the large one from which the larvae were obtained. Each rack held 64 petri dishes (8 stacks each of 8 petri dishes). Seawater in the small petri dishes was changed twice weekly, and the juveniles were fed dry, ground alfalfa that was less than 0.5 mm in diameter. The juveniles were fed immediately after the water exchange or more frequently if they required additional food. Considerable cannibalism occurred among the three juveniles in the same petri dish. In most petri dishes, only one worm survived to the juvenile stage.

When most of the juveniles had grown into young adults, their sex was determined and the females paired to vigorous males from the same brood, if sufficient males were available. If sufficient males were not available, they were paired with other males from the same dose-rate-exposure group. Next, the mated pair (first filial generation, F_1) was transferred to a large (120-mm diameter x 20-mm depth) petri dish. The petri dish with the mated pair was placed in a petri-dish rack at the same distance from the radiation source as that of the juveniles and parent worms (P_1) from which they were derived. To reduce differences in dosimetry, the petri dish containing the mated pair was always rotated so that their tube was always at the front of the rack (closest to the radiation source).

Again, the mated pairs (F_1) were observed and cared for as described for their parents (P_1). The date of spawning of the F_1 female was noted, the brood was removed from in front of the source and placed in the control area, and then the brood was sacrificed about 4 to 6 d after the spawning date. The brood was sacrificed at this time because the nurturing male consumes the dead embryos as part of taking care of the brood. Therefore, to obtain an indication of total number of embryos in the brood, the brood was sacrificed before the male had time to consume a significant number of dead embryos. In those cases when large numbers of embryos died early in development (before about 6 d), the gut of the male was yellow from yolk. When this occurred, it was recorded so that an indication could be obtained of those broods where the number spawned was greater than the number that was recorded present at the time the brood was sacrificed. The total duration of the experiment was about 8 months.

2.3 Brood Analysis

The analysis of the brood consisted of (1) enumeration and examination of the embryos and (2) a trypan-blue-exclusion test (Table 1). The analysis of the brood was performed by one or two persons. For the first part of the analysis, the embryos were removed from the tube and transferred quantitatively from their petri dish to a counting chamber, which was a petri dish bottom (60-mm diameter x 20-mm depth) that had been divided into quadrants. The counting chamber containing the embryos was placed on graph paper, and then the total number of embryos in the spawn was determined by systematically counting the embryos in each quadrant; 6X magnification was used. Next, the number of abnormal and normal embryos was evaluated at 12X

Table 1. Steps in the procedure used to harvest the broods from the F_1 mated pairs. The harvest was performed 4 to 6 d after spawning.

Part I. Enumeration and Examination.
1. Removal of developing embryos from tube to counting chamber.
2. Counting of embryos to determine brood size.
3. Determination of the stage of development of the embryos and the number of normal and abnormal embryos.
Part II. Trypan-Blue-Exclusion Test
1. Treatment of brood with trypan blue to identify living, dying, and dead embryos.
2. Preservation of embryos.
3. Calculation of estimated hatch size.

magnification. The two types of abnormal embryos identified were those that were aberrant morphologically and those that had delayed development. The morphologically abnormal embryos had atypical cleavage patterns and/or void regions (Fig. 3); the delayed-development embryos were zygotes or at the 2- or 4-cell stage when the brood was harvested. In the case where the embryos had both types of abnormalities, this fact was noted. The stages that were quantified were the unfertilized egg, zygote, 2-cell, 4-cell, prehatch, and hatchling stages; these stages were identifiable with a minimum of ambiguity. The few unfertilized eggs detected were found in broods that were scattered throughout the tube.

The second part of the brood analysis was a trypan-blue-exclusion test that was developed in our laboratory. After the embryos were counted and examined, the seawater was decanted and sufficient 0.4% trypan-blue solution in seawater to cover the embryos was added. The embryos were exposed to the trypan blue for 5 min, the excess trypan-blue solution was then decanted, and the embryos rinsed with filtered seawater until the excess blue dye was gone. The embryos were examined under 6X magnification, and the number that were totally stained blue (dead), partially stained blue (dying), and free of blue dye (live) were recorded (Fig. 4). Because of the staining of the embryos, it could not be ascertained readily whether the dead and dying embryos were normal or abnormal. Next, the seawater was decanted and 4% formalin added to preserve the embryos.

For each brood, the number of embryos that should hatch into larvae was estimated using the data on the total number of embryos compared to the number of abnormal embryos or the number of embryos that were dead or dying. In

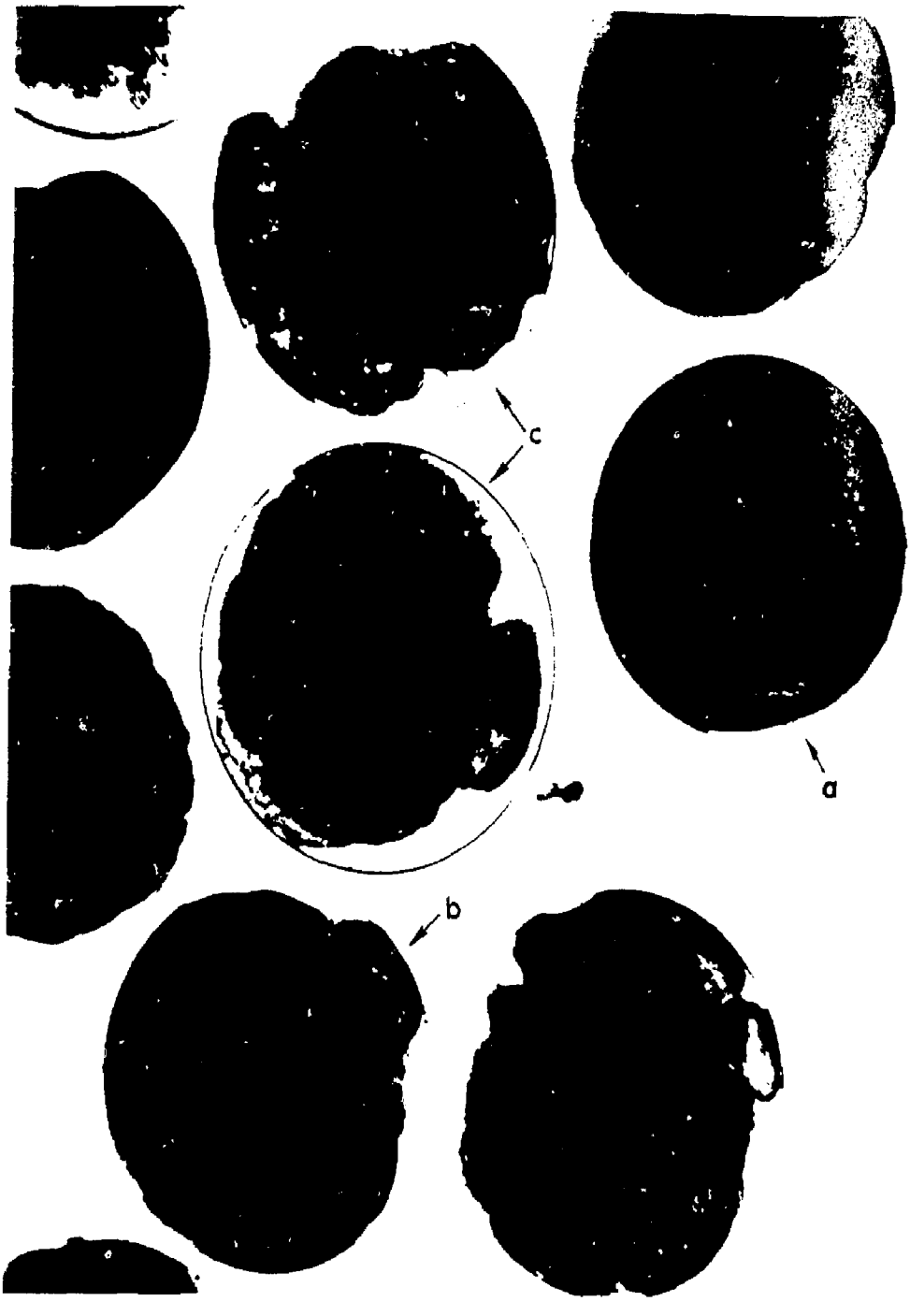

Figure 3. Embryo abnormalities identified in sacrificed broods. Normal cleavage pattern (a), atypical cleavage pattern (b), and embryos with void regions (c) are shown.

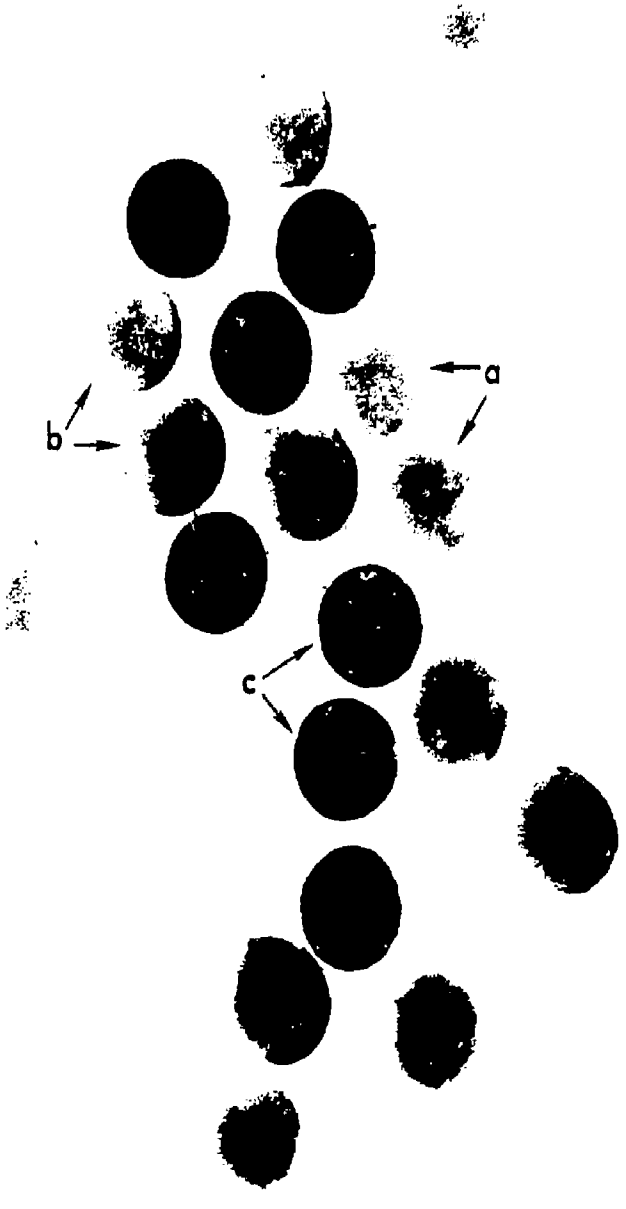

Figure 4. Broods subjected to a trypan-blue-exclusion test were differentiated into embryos that were (a) alive (free of blue color), (b) dying (partially stained blue), and (c) dead (totally stained blue).

almost all broods, the number of abnormal embryos was greater than the sum of the numbers of dead and dying embryos. The assumption made for the calculation of the hatch size was that the abnormal embryos that were living would not survive to hatching but would die and be consumed by the brooding male. The estimated hatch size (EHS) was calculated from the following relationship:

$$EHS = (\text{Total numbers in brood}) - (\text{Total number of abnormals}) .$$

For example, if the total number of embryos in the brood was 400 and if 75 were abnormal, then

$$EHS = 400 - 75$$
$$= 325 .$$

In the few cases where the number of dead and dying was greater than the number of abnormal embryos, the number of live embryos in the brood (the total number in brood minus number of dead and dying) was taken as the EHS.

Differences among control and radiation-exposed groups in brood size, in percentages of living embryos in the broods, in percentages of abnormal embryos in the broods, and in estimated and actual hatch size were analyzed using a Test for Equal Proportions (Snedecor and Cochran 1967). Also, differences in brood size for the control and radiation-exposure groups were examined using Analysis of Variance (ANOVA).

3. RESULTS

3.1 Total Doses Received

The approximate doses received by the experimental worms from the times (I) the eggs were spawned by the P_1 female until the larvae hatched and (II) the eggs were spawned by the P_1 female to the spawning of the F_1 female (normal lifespan for females) were determined (Table 2); the approximate total dose is the product of the mean duration of exposure and the mean dose rate. Because the experimental worms were shielded from the radiation source during their maintenance, their mean duration of exposure was shorter than their lifetime. The developing P_1 embryos were exposed to radiation for an average of from 10 to 12 d and received total doses that ranged from 0.055 to 4.9 Gy. The life-span dose received by the F_1 female worms ranged from 0.55 to 54 Gy.

3.2 P_1 Hatch Size

We determined the number of embryos that hatched from each of the broods of the P_1 females (Table 3). The mean number of larvae that hatched from embryos exposed to each of the different dose rates was similar to the number of control embryos that hatched. Exposure to radiation did not appear to affect the number of larvae that hatched from the P_1 broods.

Table 2. Approximate total radiation doses received by worms in each radiation-exposure group. The mean duration of the exposure is in parentheses.

Dose rate and duration	Total doses (Gy)
I. P_1 spawn to F_1 hatch	
1. 0.19 mGy/h (12 d)	0.055
2. 2.1 mGy/h (10 d)	0.50
3. 17 mGy/h (12 d)	4.9
II. F_1 life-span dose	
1. 0.19 mGy/h (120 d)	0.55
2. 2.1 mGy/h (128 d)	6.5
3. 17 mGy/h (132 d)	54

3.3 F_1 Brood Size

The numbers of F_1 mated pairs that were placed in front of the source initially were sometimes greater than the numbers for which information was obtained; some worms were lost or killed accidentally during routine maintenance (Table 3). Information about the broods was obtained for only about half the F_1 mated pairs exposed to the highest dose rate (17 mGy/h) because some females resorbed their oocytes and then died (see the Appendix).

The mean F_1 brood size was always larger than the number that hatched from the P_1 female (Table 3). Because the brood from the F_1 females was sacrificed before hatching occurred and the brood from the P_1 female was allowed to proceed to hatching, it would be expected that the total number determined for the F_1 brood would be larger than the total number of hatchlings from the P_1 female. However, there were some broods from F_1 females as small as the number of larvae that hatched from the brood of the P_1 female.

We determined the mean size of the broods from the F_1 mated pairs that were obtained from each parental brood. In the control group, brood size ranged from 6 to 637 and had a normal distribution. Each brood was distributed into one of four categories ($n \geq 150$, $150 > n \geq 100$, $100 > n \geq 50$, and $n < 50$), according to the number of embryos in the brood (Table 4). A Test for Equal Proportions was used to determine which radiation-exposed groups had brood-size distributions that were significantly different from controls. The brood-size distribution was different only for the group of worms irradiated at a rate of 17 mGy/h; the proportion of broods in the $n \geq 150$ category was lower than that of controls ($p < 0.001$). The overall mean brood size of the 0.19 and 2.1 mGy/h radiation-exposed groups did not differ

Table 3. Number of embryos from parental (P_1) and first filial (F_1) generations in control and radiation-exposed groups. The brood from the F_1 generation was sacrificed before hatching occurred.

P_1 brood ID	Parental hatch size	Breeding procedure	Mated pairs Initial	Mated pairs Final	Filial brood size ($\bar{x}$ ± SD)	
A. Control						
8-2	189	Intrabrood	26	26	188	102
15-4	95	Intrabrood	18	17	279	119
16-1	69	Intrabrood	9	9	209	70
17-5	170	Intrabrood	16	16	281	138
22-7	180	Intrabrood	11	8	252	126
24-3	211	Intrabrood	18	18	241	117
$\bar{x}$ ± SD	152 ± 58				238	118
B. 0.19 mGy/h						
1-2	192	Interbrood	7	7	216	61
5-1	48	Interbrood	6	6	226	104
25-3	150	Interbrood	18	18	169	64
26-4	126	Interbrood	17	17	210	90
27-5	72	Interbrood	9	9	215	96
29-7	81	Interbrood	21	17	244	93
31-8	100	Interbrood	12	10	211	92
$\bar{x}$ ± SD	110 ± 50				211	92
C. 2.1 mGy/h						
11-4	11)	Intrabrood	14	14	201	97
16-2	120	Intrabrood	14	14	218	68
20-1	93	Intrabrood	6	5	215	70
21-3	81	Interbrood	14	14	249	85
23-5	43	Intrabrood	8	8	238	95
24-6	150	Interbrood	13	13	222	55
27-8	180		12	12	253	123
$\bar{x}$ ± SD	112 ± 45				227	88
D. 17 mGy/h[a]						
4-1	111	Interbrood	23	22	78	114
10-4	120	Intrabrood	22	21	133	148
11-2	120	Intrabrood	16	16	177	158
$\bar{x}$ ± SD	117 ± 5				124	142

[a] Females that resorbed their eggs and then died were included in the compilation as having a brood size of zero.

Table 4. Number of embryos in broods from the control and radiation-exposed mated pairs. The broods were sacrificed before hatching occurred and were assigned to one of four categories ($n \geq 150$, $150 > n \geq 100$, $100 > n \geq 50$, and $n < 50$), according to the number of embryos in the brood.

Experimental group	Categories of numbers of embryos in broods				Total broods
	$n \geq 150$	$150 > n \geq 100$	$100 > n \geq 50$	$n < 50$[a]	
	A. Number of broods in category				
Control	73	10	9	2	94
0.19 mGy/h	64	12	6	2	84
2.1 mGy/h	70	6	3	1	80
17 mGy/h	23	6	4	26	59
					317
	B. Percent of broods in category				
Control	77.7	10.6	9.6	2.1	94
0.19 mGy/h	76.2	14.3	7.1	2.4	84
2.1 mGy/h	87.5	7.5	3.8	1.2	80
17 mGy/h	39.0	10.2	6.8	44.1	80
					317

[a] Females that resorbed their eggs and then died were included in the compilation as having a brood size of zero.

significantly from that of the control group, but that of the 17 mGy/h group did (one way ANOVA $F = 15.04$, $p < 0.0001$). The group receiving 17 mGy/h was significantly different from the controls because 25 of the 59 females resorbed their eggs and then died at approximately the time of spawning, and these females were included in the compilation as having a brood size of zero. These data indicate that these levels of radiation, which were received over the lifetime of the female worms and ranged from about 0.6 to 6.5 Gy, did not result in a reduced number of F_2 embryos in the brood.

3.4 Living Embryos in F_1 Broods

For each brood from a F_1 mated pair, the percentage of the F_2 embryos that were living (as evidenced by the exclusion of trypan blue from their cells) was calculated for the group of control worms and for each of the groups of worms that were exposed to one of the three dose rates of radiation. The percentages, which were distributed into four categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, and $n < 25\%$), were related to the dose rate received. For the control group, almost all the developing F_2 embryos in the broods were living. Of the 90 control broods, 78 of these broods were in the $n \geq 75\%$ category; stated as a percentage, 86.7% of the control broods were in the $n \geq 75\%$ category (Table 5). In contrast, the percentage of the broods in which $n \geq 75\%$ of the embryos were living in the 0.19 mGy/h group was 62.1; in the 2.1 mGy/h group was 49.3; and in the 17 mGy/h group was 3.4.

Table 5. Results from the trypan-blue-exclusion test of the living, dying, and dead F_2 embryos in the broods from the F_1 mated pairs. The broods were sacrificed before hatching occurred and were assigned to one of four percentage categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, and $n < 25\%$), according to the percentage of living embryos in the brood.

Experimental group	Categories of percentages of living embryos in broods				Total broods
	$n \geq 75$	$75\% > n \geq 50\%$	$50\% > n \geq 25\%$	$n < 25\%$[a]	
	A. Number of broods in category				
Control	78	5	1	6	90
0.19 mGy/h	36	13	5	4	58
2.1 mGy/h	34	16	12	7	69
17 mGy/h	2	5	5	47	59
					276
	B. Percent of broods in category				
Control	86.7	5.6	1.1	6.7	90
0.19 mGy/h	62.1	22.4	8.6	6.9	58
2.1 mGy/h	49.3	23.2	17.4	10.1	69
17 mGy/h	3.4	8.5	8.5	79.6	59
					276

[a] Females that resorbed their eggs and then died were included in the compilation as broods with n = 0% living embryos.

The results from the trypan-blue-exclusion test indicate that with increased dose rate there is a decreased percentage of living embryos in the brood (Fig. 5). Using the Test for Equal Proportions, we determined that the number of broods in the $n \geq 75\%$ category for the group of worms exposed to 0.19 mGy/h was significantly different from the number in that category for the group of control worms; $X^2 = 12.06$, $p < 0.001$. The proportion of the broods that was in the $n \geq 75\%$ category for each of the other more intensely radiated groups was also significantly different from that of the control group ($p < 0.001$). These results indicate that, for this species, a lifetime dose rate as low as 0.19 mGy/h or a total dose of about 0.6 Gy (60 rad) reduces significantly the percentage of living embryos in the brood.

The brooding males are effective at removing dead embryos from the brood. This is evident from the data acquired on the broods in which the embryos hatched into larvae before they were analyzed (see comment section of brood data in the Appendix). When hatching did occur, the percentage of living embryos almost always approached 100. If large numbers of early-stage embryos are eaten by the male, his gut is yellow from the yolk consumed; these

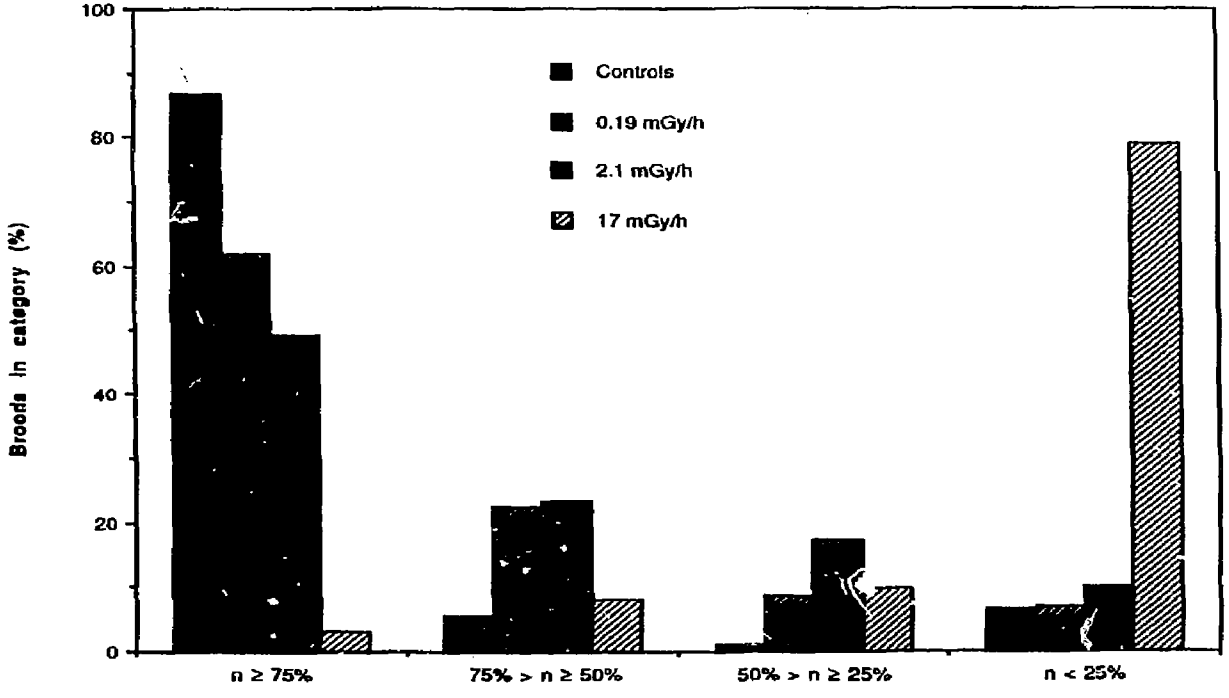

Figure 5. The percent of broods from the F_1 mated pairs in each of four categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, and $n < 25\%$) of percent living embryos in the brood.

males are referred to as cannibals. A few males, even in the control group, cannibalized the brood, but at the higher dose rates this was a common occurrence, presumably because there were more dead embryos present. The percentage of the males that were cannibals was 17 in the control group, 27 in the 0.19 mGy/h group, 24 in the 2.1 mGy/h group, and 83 in the 17 mGy/h group.

3.5 Abnormal Embryos in F_1 Broods

In most broods, some embryos were classified as abnormal because of their morphology or because their development was delayed severely. The broods were placed into four categories ($n \geq 150$, $150 > n \geq 100$, $100 > n \geq 50$, and $n < 50$), according to the number of abnormal embryos in the brood (Table 6, Fig. 6). The percent of the broods in the $n < 50$ category was 80.2 for the control group and was 60.7, 35.3, and 5.1 for the groups exposed to 0.19, 2.1, and 17 mGy/h, respectively. We also calculated the percentages of abnormal embryos that were present, and these were distributed into four categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, and $n < 25\%$)(Table 7, Fig. 7). The percent that was in the $n \geq 75\%$ category was 1 for the control group and 7, 16, and 91 for the groups exposed to 0.19, 2.1, and 17 mGy/h, respectively. Incidence of abnormal embryos appears to be dose related. A significant difference from the control group was detected in all the radiation-exposed groups. For the group exposed to 0.19 mGy/h, $X^2 = 6.66$, $p < 0.005$.

3.6 Reduced Survival of F_1 Embryos

The numbers of embryos that were estimated to hatch or the actual numbers that hatched were grouped into four categories: ($n \geq 150$, $150 > n \geq 100$, $100 > n \geq 50$, and $n < 50$) (Table 8). The hatch size was related to dose rate (Fig. 8). The percent of the broods that had or were estimated to have hatchlings ≥ 150 in number was 68.1 for the control group and was 50.0, 36.3, and 0 for the radiation-exposed groups receiving 0.19, 2.1, and 17 mGy/h, respectively. Also, the estimated size of the hatch from the F_1 mated pairs exposed to radiation was significantly different from that of controls for all the lifetime dose rates delivered to the worms.

The effects of radiation were apparent also in the percentage of broods in which the EHS was zero. The percentage was 1.2 for the control group and was 5.4, 7.7, and 42.3 for the groups exposed to 0.19, 2.1, and 17 mGy/h, respectively. An EHS of zero resulted because the female resorbed the eggs or because the embryos in the brood were either abnormal, dead, or dying.

The effects of radiation on the potential for embryos to survive to hatching was assessed. The percent of the embryos that should survive to hatching for each brood was calculated by dividing the EHS by the brood size and multiplying the fraction by 100. Then, the broods were assigned to one of four categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, and $n < 25\%$), according to the percentages of survival (Table 9, Fig. 9). The Test for Equal Proportions was used to determine which radiation groups had distributions of percentages that were significantly different from that of the controls. All groups exposed to radiation were significantly different from controls; the p values were < 0.001.

Table 6. Results from the analysis of the normal and abnormal embryos in the broods from the control and radiation-exposed F_1 mated pairs. The broods were sacrificed before hatching occurred and were assigned to one of four categories ($n \geq 150$, $150 > n \geq 100$, $100 > n \geq 50$, and $n < 50$), according to the number of abnormal embryos in the brood.

Experimental group	Categories of numbers of abnormal embryos in broods				Total broods
	$n \geq 150$	$150 > n \geq 100$	$100 > n \geq 50$	$n < 50$[a]	
	A. Number of broods in category				
Control	4	7	7	73	91
0.19 mGy/h	5	6	11	34	56
2.1 mGy/h	17	10	17	24	68
17 mGy/h	47	4	5	3	59
					274
	B. Percent of broods in category				
Control	4.4	7.7	7.7	80.2	91
0.19 mGy/h	8.9	10.7	19.6	60.7	56
2.1 mGy/h	25.0	14.7	25.0	35.3	68
17 mGy/h	79.6	6.8	8.5	5.1	59
					274

[a] Females that resorbed their eggs and then died were included in the compilation as broods in the $n \geq 150$ category.

An analysis was performed to determine the relationship between chronic radiation dose and embryo survival. The mean percent survival for the control group and for each radiation-exposed group was determined. For the control group, a value of 82 ± 18% was obtained, and for the groups exposed to radiation, the values were 61 ± 28% for the group exposed to 0.19 mGy/h, 51 ± 31% for the group exposed to 2.1 mGy/h, and 5 ± 13% for the group exposed to 17 mGy/h. The mean percentage for each radiation-exposed group was expressed also as a percentage of the control group. A semilog plot of percentages versus dose resulted in a straight line; an LD_{50} of about 10 Gy and an LD_{99} of about 100 Gy was obtained (Fig. 10).

Other parameters that were examined for the experimental animals were (1) the time from spawning of the P_1 brood to the hatching of the F_1 larvae (spawn-to-hatch time) and (2) the time from the hatching of the larvae (F_1) until the spawning of the adult females (F_1) (hatch-to-spawn time). The mean spawn-to-hatch time for all the P_1 broods was 11.7 ± 1.8 d, and the irradiated groups did not differ significantly from controls. These data indicate that

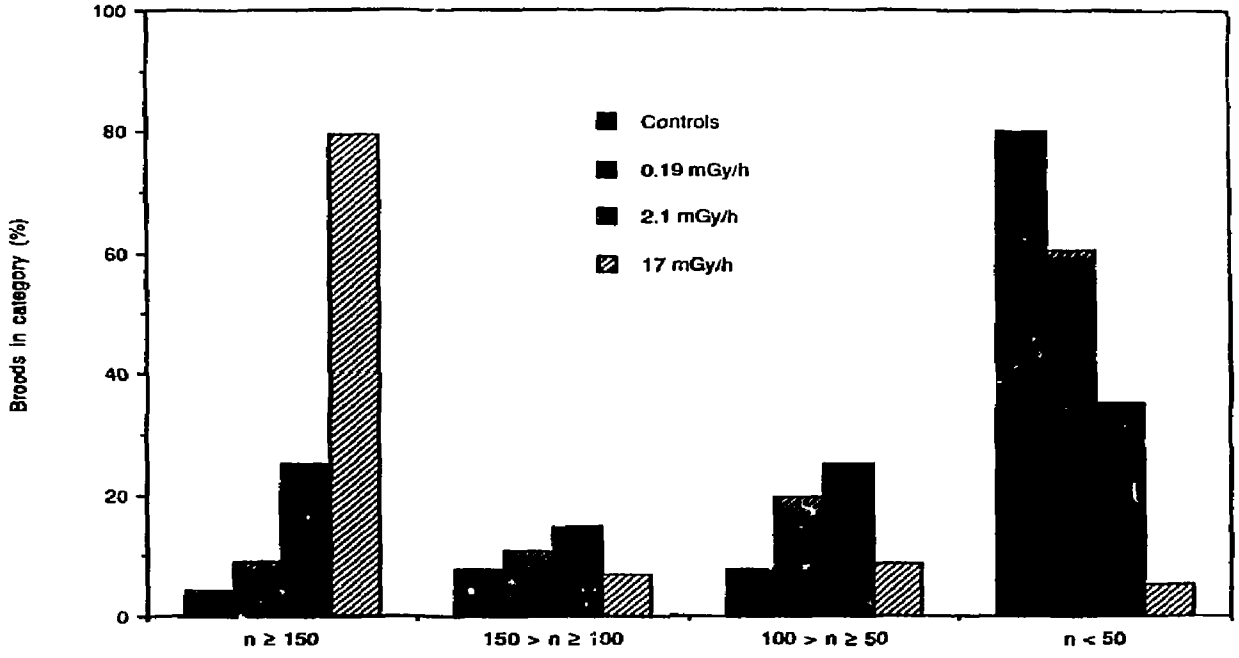

Figure 6. The percent of broods from F_1 mated pairs in each of four categories ($n \geq 150$, $150 > n \geq 100$, $100 > n \geq 50$, and $n < 50$) of numbers of abnormal embryos in the brood.

Table 7. Results from the analysis of normal and abnormal embryos in the broods from the control and radiation-exposed mated pairs The broods were sacrificed before hatching occurred, the number of normal and abnormal embryos determined, the percent of abnormal embryos calculated, and then the broods were assigned to one of four categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, $n < 25\%$), according to the percent of abnormal embryos in the brood.

Experimental group	Categories of percentages of living embryos in broods				Total broods
	$n \geq 75\%$	$75\% > n \geq 50\%$	$50\% > n \geq 25\%$	$n < 25\%$[a]	
	A. Number of broods in category				
Control	1	2	9	77	89
0.19 mGy/h	4	15	15	23	57
2.1 mGy/h	11	19	16	22	68
17 mGy/h	54	4	1	0	59
					273
	B. Percent of broods in category				
Control	1.1	2.2	10.1	86.6	89
0.19 mGy/h	7.0	26.3	26.3	40.4	57
2.1 mGy/h	16.2	27.9	23.5	32.4	68
17 mGy/h	91.5	6.8	1.7	0	59
					273

[a] Females that resorbed their eggs and died were included in the compilation as broods with 100% abnormal embryos.

when worms were irradiated with doses as high as 17 mGy/h and were given a total dose of 4.9 Gy during the spawn-to-hatch time, the time required to develop from fertilized eggs to larvae was not affected. The mean spawn-to-harvest time for all the F_1 females was 127 ± 18 d, and there were no significant differences among experimental groups. These data indicate that radiation at doses as high as 17 mGy/h and mean total doses of about 54 Gy also did not affect the life span of the females.

4. DISCUSSION

Living organisms are exposed to radiation from natural sources and from anthropogenic sources, including nuclear explosions, routine and accidental releases from nuclear power facilities, and nuclear waste disposal (UNSCEAR 1977, 1982). The dose rates to marine organisms from natural background radiation, global fallout, and waste radionuclides were calculated by Woodhead (1984) and provide a perspective within which the possible harmful effects of increased radiation exposure can be considered. The dose rates in the marine environment due to radionuclide inputs arising from human activities range from less than the natural background exposure for typical nuclear power stations in routine operations up to a few tenths of mGy/h for the rather exceptional case of the Windscale discharge into the northeast Irish Sea (Woodhead 1984).

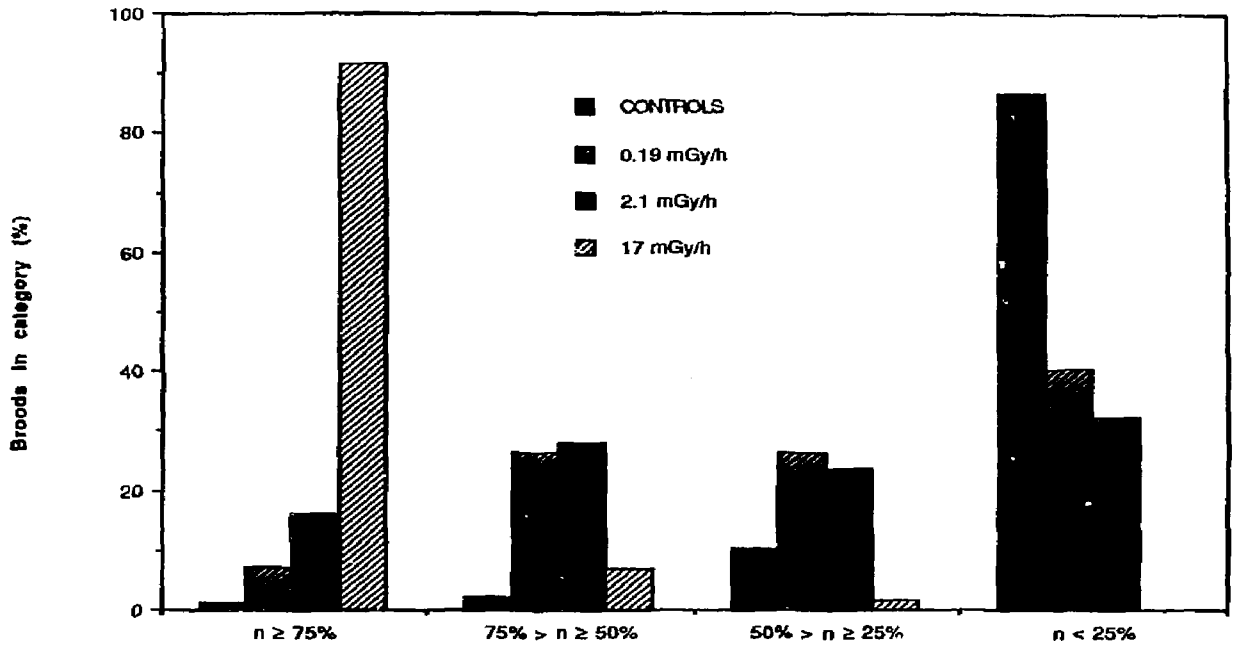

Figure 7. The percent of broods in each of four categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, and $n < 25\%$) of percent abnormal embryos in the brood.

Table 8. Results from the analysis of the numbers of F_2 embryos that actually hatched or were estimated to hatch from the broods of the control and radiation-exposed F_1 mated pairs. The broods were assigned to one of four categories ($n \geq 150$, $150 > n \geq 100$, $100 > n \geq 50$, and $n < 50$), according to the actual or estimated hatch size.

Experimental group	Categories of numbers estimated or actually in hatches				Total broods
	$n \geq 150$	$150 > n \geq 100$	$100 > n \geq 50$	$n < 50$	
	A. Number of broods in category				
Control	64	12	10	8	94
0.19 mGy/h	42	18	14	10	84
2.1 mGy/h	29	20	14	17	80
17 mGy/h	0	1	3	57	61
					319
	B. Percent of broods in category				
Control	68.1	12.8	10.6	8.5	94
0.19 mGy/h	50.0	21.4	16.7	11.9	84
2.1 mGy/h	36.3	25.0	17.5	21.3	80
17 mGy/h	0	1.6	4.9	93.4	61
					319

It is well documented that radiation induces biological effects through the deposition of energy in the cells of the irradiated individuals (UNSCEAR 1982). If the effects are produced in the somatic cells, they must become apparent, by definition, within the life of the irradiated organism. If the effects are produced in the germ cells, whose function is to transmit genetic information to new individuals, the effects may be detected in the descendants of the irradiated individual in the first or subsequent generations.

Most of the information available on radiation effects on reproductive success in aquatic animals is on the effects of acute radiation. Effects were determined by irradiating early life stages and adults (see reviews by Egami and Ijiri 1979; Woodhead 1984; Anderson and Harrison 1986). The effects of acute radiation on processes affecting reproductive success in aquatic invertebrates were reported for doses that range over at least two orders of magnitude (Cervini and Giavelli 1965; Ravera 1967; Hoppenheit 1973; Greenberger et al. 1986; Anderson et al. 1987). Causes for this broad range seem to be not only actual species-specific differences in gamete sensitivity, but also differences in the gamete stage irradiated and in the cell-repopulation capacity of different organisms.

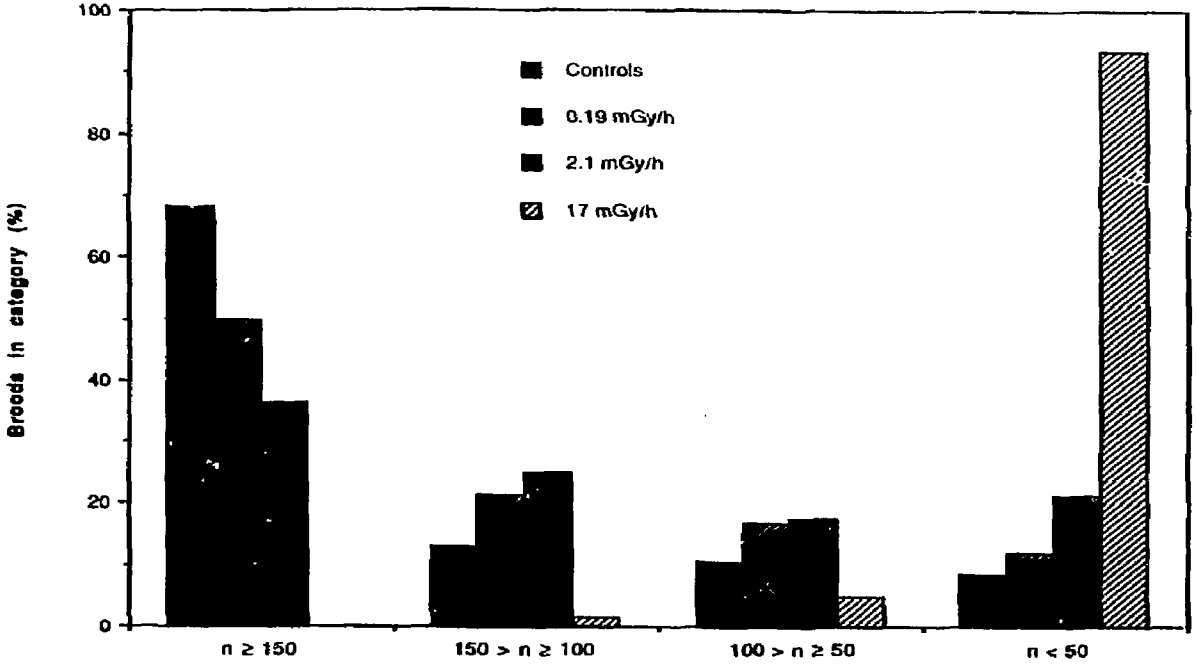

Figure 8. The percent of broods from the F_1 mated pairs in each of four categories ($n \geq 150$, $150 > n \geq 100$, $100 > n \geq 50$, and $n < 50$) of actual and estimated numbers of hatchlings.

Table 9. Results of the analysis of survival to hatching of embryos in the broods of the control and radiation-exposed F_1 mated pairs. The percent survival was calculated by dividing the estimated hatch size by the brood size, and then the broods were assigned to one of four categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, and $n < 25\%$), according to the percent survival of the embryos.

Experimental group	Categories of percent survival of embryos to hatching				Total broods
	$n \geq 75\%$	$75\% > n \geq 50\%$	$50\% > n \geq 25\%$	$n < 25\%$[a]	
	A. Number of broods in category				
Control	68	9	2	2	81
0.19 mGy/h	20	17	13	5	55
2.1 mGy/h	21	13	18	13	65
17 mGy/h	0	1	3	47	51
					252
	B. Percent of broods in category				
Control	84.1	11.0	2.5	2.5	81
0.19 mGy/h	36.4	30.9	23.6	9.1	55
2.1 mGy/h	32.3	20.0	27.7	20.0	68
17 mGy/h	0	2.0	5.9	92.1	51
					252

[a] Data from broods that hatched or that were harvested before day 3 were excluded.

Studies were conducted to assess the effects of chronic low-level radiation on reproduction in fishes and invertebrates, and a number of these were conducted over a full life cycle. However, most of the experiments to assess the effects of chronic radiation were performed using radionuclides in the water and the doses delivered were uncertain (Woodhead 1984; Anderson and Harrison 1986).

Information on the effects of chronic radiation on reproductive success in fishes and aquatic invertebrates is available from studies in which the effects of relatively low dose rates were investigated. Trabalka and Allen (1977) compared populations of the mosquitofish Gambusia affinis from the radionuclide-contaminated White Oak Lake at the Oak Ridge National Laboratory to those from a matched control site. They found no decrease in fecundity, but an increase in embryo mortality of the fish from White Oak Lake; these fish received about 0.25 mGy/h. These results were confounded by the fact that contaminants other than radionuclides were present in White Oak Lake. Cooley (1973) examined the reproductive biology of pond

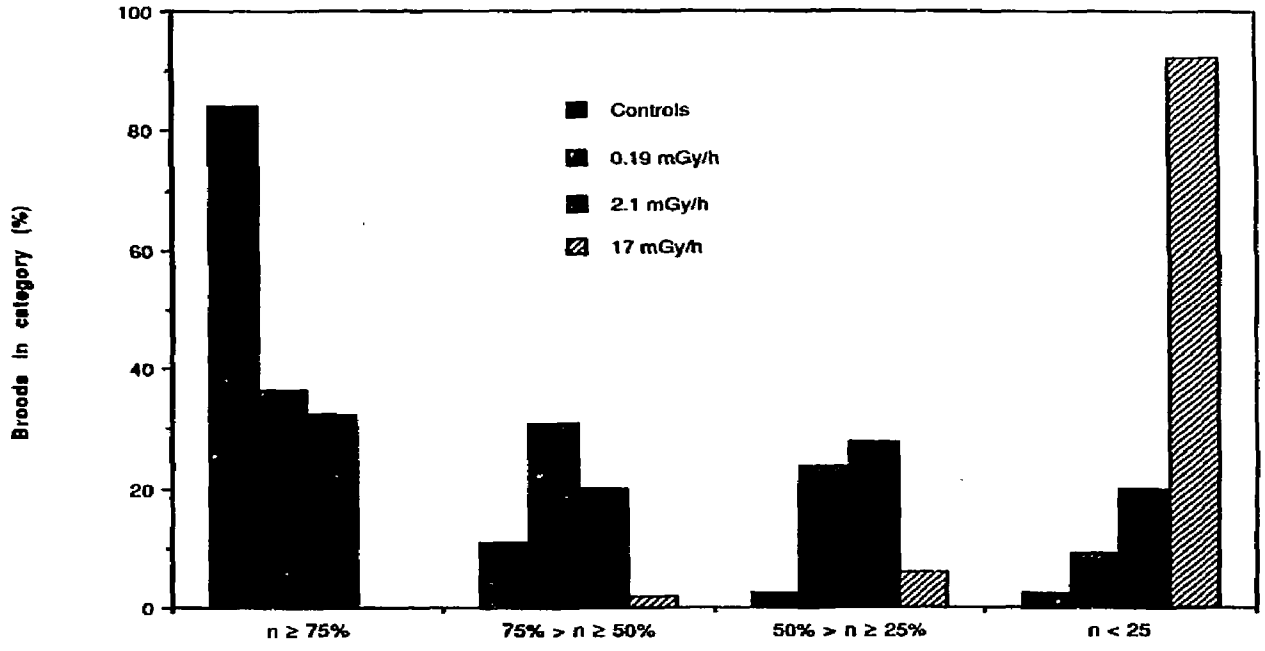

Figure 9. The percent of broods in each of four categories ($n \geq 75\%$, $75\% > n \geq 50\%$, $50\% > n \geq 25\%$, and $n < 25\%$) of percent survival to hatching of the embryos in the brood.

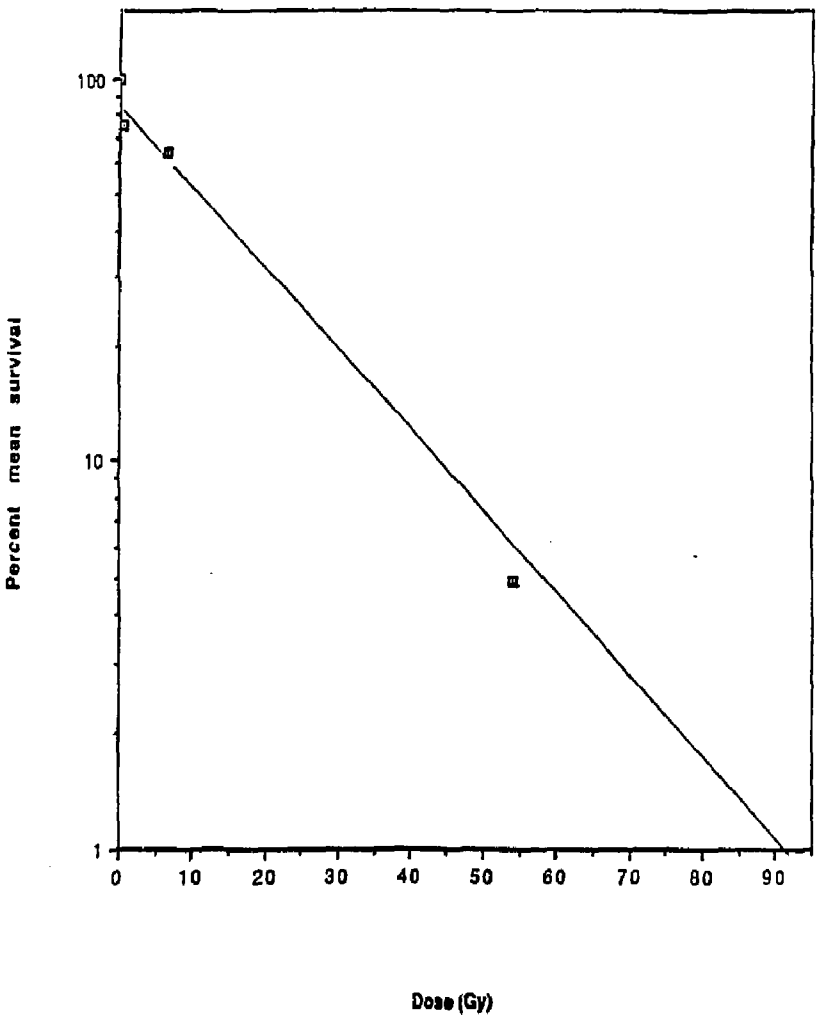

Figure 10. Mean percent survival of embryos (expressed as percentage of the survival fraction of the controls) as a function of chronic dose. Data from broods that hatched or that were harvested before day 3 were excluded.

snails from White Oak Lake; these were exposed to about the same dose rate as the mosquitofish. He found that frequency of egg-capsule production was reduced; however, an increased number of eggs per capsule was also documented. It is interesting to note that a prior laboratory study by Cooley and Miller (1971) documented a clear cut reproductive decline at 240 rad/d (100 mGy/h) but not at 24 rad/d. Irradiation was initiated on 45-d-old snails, and laboratory effects might have been observed at lower levels if irradiation had been extended over the entire lifetime of the organism.

One of the most rigorous studies involving chronic exposure to radiation was that of Woodhead (1977), who examined fecundity of the guppy Poecilia reticulata receiving 4.08, 9.6, and 30.5 rad/d (1.7, 4.0, and 12.7 mGy/h). Total fecundity was significantly reduced at all dose rates. Reductions in fecundity were probably due to both reproductive effects (damage to gametes) and the induction of dominant-lethal mutations in gametes. Effects on gonadal cells were reported also for Gambusia affinis (Cosgrove and Blaylock 1973) and for aryzias latipes (Hyodo-Taguchi and Egami 1977; Hyodo-Taguchi 1980). Hyodo-Taguchi (1980) observed an increased percentage of unfertilized Oryzias latipes eggs after males used to inseminate the eggs received approximately 6.9 rad/d (2.9 mGy/h) for 60 d. No statistically significant effects were observed at 2.9 rad/d, the next lower dose rate used. Bonham and Donaldson (1972) exposed Chinook salmon Oncorhynchus tschawytscha embryos for the first 80 d of life to 0.19 to 17 R/d (about 0.08 to 6.8 mGy/h). Approximately 4 wk after the irradiations were completed, gonadal development was observed in smolts. They found that gonadal development was retarded in those receiving at least 10 R/d.

In a more recent study, Rackham and Woodhead (1984) examined the effects of chronic gamma irradiation on the gonads of the adult fish Ameca splenden. The dose rate used was 7.3 mGy/h; after an accumulated dose of 0.95 Gy, spermatogenesis was disrupted, and after an accumulated dose of 9.7 Gy, there was no further production of sperm.

It is apparent from the data available that direct comparisons of sensitivity among species irradiated chronically are often not valid because the duration of the radiation differed from partial to several lifetimes. Research on effects of chronic radiation on the gonads is of particular interest, however, because the results show effects levels comparable to those observed in mammals. Dose rates between 0.2 and 4 mGy/h appear to define a critical range in which detrimental effects on processes contributing to reproductive success are first observed in a variety of sensitive organisms.

In our study, the effects of lifetime radiation on reproductive success of a relatively low fecundity species were evaluated. Information was obtained on the effects of chronic radiation on total number of developing embryos in the brood, on the numbers of normal and abnormal embryos in the brood, on the numbers of embryos that were living, dying, and dead, and on the estimated number of hatchlings. Comparisons were made of the data from control worms and from worms that were exposed to radiation immediately after fertilization occurred until they released their gametes and the next generation of zygotes were formed. Thus, germ cells were irradiated from their time of origin (primordial germ cells) until mature gametes were released.

An important effect of lifetime irradiation of N. arenaceodentata with low dose rate (0.19 and 2.1 mGy/h) was increased mortality of the embryos (F_2 generation). There was no evidence for F_1 gamete death or for reduced fertilization success because the number of developing embryos in the broods did not decrease. However, at the highest dose rate used, 17 mGy/h, brood size was affected and was related to the resorption of oocytes in the females. Also, at all three dose rates used, there was no detectable effect on the time required for the fertilized eggs to develop into larvae or on the life span of the female F_1 worm.

Increased mortality of the F_2 embryos was indicated because both the number of dead and dying embryos and the number of abnormal embryos found in the brood after 4 to 6 days of development increased with increased dose rate. Both of these factors contributed to a decreased number of actual or estimated number of hatchlings in the broods and occurred at the lowest rate used, 0.19 mGy/h. The increased mortality was most likely from the induction of lethal mutations in the germ cells during gametogenesis. Because both the males and females were given lifetime irradiation and because little is known about the comparative sensitivity of cells in the different stages of oogenesis and spermatogenesis in N. arenaceodentata, it is not known whether the lethal mutations occurred primarily during oogenesis, spermatogenesis, or relatively equally during both of these processes.

Effects of acute radiation on reproduction of N. arenaceodentata were examined in a companion study (Harrison and Anderson 1988), and comparisons of the effects on reproduction of total doses received from acute and chronic radiation were made (Table 10). For the parameters compared, the control group for the worms irradiated acutely appeared to be less vigorous than for those irradiated chronically; there was a greater proportion of small broods, fewer living embryos, etc. The differences between the two control groups may have been due to differences in their maintenance conditions. For the experiment in which the mated pairs were irradiated chronically, the broods were from females that were raised in our laboratory under uniform conditions of food availability and temperature whereas for those irradiated acutely, the females were from multiple sources and may not have been raised under similar conditions.

Effects on brood size, which may be due to oocyte killing, were seen when a total dose of 50 Gy was given over the lifetime of the female and when an acute dose of 10 or 50 Gy was given at the time oocytes were visible in the coelom. Information available from the mouse indicates that the target for cell killing and that for genetic effects are different and distinct in this species; the lethality target in immature oocytes appears to be the plasma membrane and the sensitivity of this target differs almost two orders of magnitude with stage in the mouse life cycle (Straume et al. 1987; Straume et al. 1988). For N. arenaceodentata, we do not have sufficient radiobiological information to evaluate the effect of developmental stage on oocyte radiosensitivity.

Comparison of the values (except brood size) that were corrected for controls shows that for those broods from females receiving a total dose of about 0.5 or 5 Gy, the effects were similar or greater for those irradiated acutely (Table 10). However, the differences between the effects elicited by

Table 10. Comparison of the effects of acute and chronic irradiation on Neanthes arenaceodentata. The values are percents of the broods in the category indicated.

Effects	Acute Control	Chronic Control	Acute 0.5 Gy	Chronic 0.55 Gy	Acute 5.0 Gy	Chronic 6.5 Gy	Acute 50 Gy	Chronic 54 Gy
Brood size[a]	10	2	15	2	26	1	56	44
(n < 50 category)			(5)	(0)	(16)	(0)	(46)	(42)
Living embryos[b]	57	87	31	62	22	49	14	3
(n ≥ 75% category)			(54)	(71)	(39)	(56)	(25)	(3)
Abnormal embryos[a]	18	1	25	7	38	16	71	91
(n ≥ 75% category)			(7)	(6)	(20)	(15)	(53)	(90)
Estimated hatch size[a]	23	8	38	12	50	21	82	93
(n < 50 category)			(15)	(4)	(27)	(13)	(59)	(85)
Survival to hatching[a]	20	2	29	9	40	20	73	92
(n < 25% category)			(9)	(7)	(20)	(18)	(53)	(90)
Survival of embryos[b]	60	82	48	61	39	52	20	5
(mean percent)			(80)	(74)	(65)	(63)	(33)	(6)

[a] Values in parentheses are minus the control values or are expressed as percents of the control value.
[b] Values in parentheses are expressed as percents of the control value.

Table 11. Comparison of the effects on reproductive success of exposure of Neanthes arenaceodentata to different doses of contaminants.

	Ionizing radiation (Gy)		Hexavalent chromium[a] (µg/L)	Number 2 fuel oil[b] (%WSF)
Response	Acute	Chronic	Chronic	Chronic
Sterility	50[c]	90[c]	100	--
Reduction in number of embryos	10	54	--	2.5
Reduction in number of hatchlings	0.5	0.55	16-38	2.5

[a] Oshida and coworkers (1981, 1982).
[b] Rossi and Anderson (1978); WSF is the water-soluble fraction.
[c] Effective sterility is defined here as 1% survival of embryos to hatching as compared to controls.

radiation given acutely and that given chronically was less than was expected. These results indicate that there was most likely accumulation of radiation damage in nondividing cells and, then, this damage became apparent after fertilization when the cells started to divide. This finding is of special interest because such damage accumulation may occur not only with the direct-acting mutagen, radiation, but also with other direct- and indirect-acting organic mutagens that may be present in ecosystems. Although we have no direct evidence for such, the damage accumulation may be related to differences in DNA-repair ability of cells in different stages in gametogenesis.

Comparison of the values (corrected for controls) for females receiving a total dose of about 50 Gy acutely and chronically indicates that the effects appear to be more severe in those irradiated chronically. There are two plausible explanations for this response. First, all gametogenic stages are irradiated during chronic exposures and a particular stage of oocyte development may be sensitive to high dose rates. This could be relatively short hypersensitive stage that is only "hit" by chronic radiation. Second, an unknown radiation-induced stress may have been induced at the high dose rate, and this stress may have caused resorption of the oocytes prior to spawning. The overall effect would be reduced fecundity.

Evidence that the oocytes are the limiting cell system was obtained from a comparison of the data from the preliminary and final experiments on acute effects of radiation. In the preliminary experiment, only the females were irradiated (Anderson et al. 1987), while in the final experiment, mated pairs

(male and female) were irradiated (Harrison and Anderson 1988). The results of both the preliminary and the final experiments were similar; this indicates that the oocytes were most likely the cells in which radiation damage was accumulated.

Information available on mammals indicates that in some species the oocytes are very sensitive to radiation (UNSCEAP 1986; Dobson et al. 1984; Dobson et al. 1986). Sensitive species include mice and some primates. In the mouse, the LD_{50} for immature-oocyte killing with ^{60}Co gamma rays is 1.75 Gy in the prenatal mouse and range from about 0.05 to 0.15 Gy in the juvenile mouse. The value of 0.05 Gy for juvenile mice reflects about a 30 to 50 times greater sensitivity than found in most other cells studied. In the squirrel monkey, the LD_{50} for radiation from administering tritiated water was 0.07 Gy from prenatal exposure and 2.25 Gy from adult exposure. It is apparent that there are considerable differences in sensitivity with species and life stage. In N. arenaceodentata, we know little about differences in sensitivity with defined life stages. However, from the results we obtained, it appears that a dose at least 10 times higher is required to affect cell killing in N. arenaceodentata than in least sensitive stage of the mouse, but that the sensitivity of N. arenaceodentata is in the range of most other cells studied.

Little is known about the effects of factors that may modify the responses of aquatic organisms to radiation. Factors that may play an important role are DNA repair, tissue oxygen concentrations, and environmental conditions, such as temperature, salinity, and water quality (Anderson and Harrison 1986). Some of these factors are known to modify the responses of vertebrates to radiation and require elucidation before conclusions are drawn about regulatory limits on the quantities of mutagens released in the environment.

Information is available for N. arenaceodentata on the effect on reproductive success of contaminants other than radiation (Table 11). Considerable data are available on the effects of chromium (Oshida et al. 1981; Oshida and Ward 1982). Concentrations of chromium as low as 16 µg/L reduced the numbers of hatchlings. The concentration of chromium that resulted in sterility was 100 µg/L. However, sterility occurred not because of effects on gametes but because of a behavioral response of the adult worms. According to these investigators, the worms were jerking and twisting to such an extent that the prolonged contact required for reproduction did not occur.

The water-soluble fraction (WSF) of Number 2 fuel oil also impacted on reproduction in N. arenaceodentata (Rossi and Anderson 1978). Effects on the number of larvae that hatched occurred at concentrations as low as 2.5% WSF (Table 11). No information was available on the WSF concentrations resulting in sterility, but growth was inhibited at 5 and 10% WSF.

The studies of the effects of both chromium and fuel oil were multigeneration and provided evidence that there was accommodation to the contaminants in the F_2 and F_3 generations. Because our study of radiation effects was only for a single generation, no conclusions can be drawn as to possible accommodation by subsequent generations or to the response of populations to continuous exposure to low levels of radiation.

There are few data on chronic radiation effects on invertebrates that can be compared to those reported here on N. arenaceodentata. However, it is apparent from the data available on fish and invertebrates that the overall effects on reproductive success are dependent upon a number of factors. Important among these are reproductive strategy and sensitivity of stages in gametogenesis and in early development. It would be expected that species most vulnerable to chronic exposures to low levels of mutagenic contaminants are those that have a low fecundity and have highly sensitive stages. Because the results from our study indicate that in some invertebrates the range of sensitivity may overlap with that for fish and even for mammals and because the data base on effects of chronic low-level exposures is limited, it may not be overly conservative to adopt limits for the chronic exposure of low-fecundity aquatic animals based on the extensive data base available on the responses of mammals.

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APPENDIX

Data Base from the Experiment to Determine the Effects of Chronic Radiation on Reproductive Success of *Neanthes arenaceodentata*

Table A-1. Experimental data from Neanthes arenaceodentata mated pairs that were not irradiated with an external gamma-radiation source (controls). The number of days from spawn to hatch and from hatch to spawn as well as the estimated hatch number are provided.

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
12	119	8-2-1	73	57	78.1	16	0	0	5	57	Can[a]
12	125	8-2-2	226	213	94.2	11	2	0.9	60	166	Can
12	129	8-2-3	452	434	96.0	11	7	1.6	2	434	Can
12	129	8-2-6	103	101	98.1	2	0	0	15	88	
12	128	8-2-10	307	304	99.0	1	2	0.7	62	245	
12	119	8-2-10b	91	91	100	0	0	0	1	90	Hatch[b]
12	134	8-2-16	361	357	98.9	2	2	0.6	21	340	
12	128	8-2-32	202	200	99.0	0	2	1.0	15	187	
12	134	8-2-36	202	202	100	0	0	0	18	184	
12	122	8-2-37	96	93	96.9	2	1	1.0	3	93	
12	121	8-2-42	230	205	89.1	20	5	2.2	24	205	
12	121	8-2-49	115	112	97.4	3	0	0	10	105	
12	152	8-2-52	38	34	89.5	2	2	5.3	0	34	Can
12	119	8-2-53	88	74	84.1	14	0	0	11	74	Can
12	149	8-2-55	327	325	99.4	1	1	0.3	22	305	
12	136	8-2-56	121	121	100	0	0	0	0	121	Scat
12	128	8-2-60	223	203	91.0	17	3	1.4	40	183	
12	121	8-2-63	165	157	95.2	2	6	3.6	16	149	
12	134	8-2-66	306	305	99.7	0	1	0.3	25	281	
12	125	8-2-68	196	185	94.4	5	6	3.1	52	144	
12	119	8-2-74a	218	217	99.5	1	0	0	18	200	
12	125	8-2-74b	239	229	95.8	8	2	0.8	47	192	
12	124	8-2-75	209	205	98.1	3	1	0.5	21	188	
12	120	8-2-77a	111	98	88.3	12	1	0.9	21	90	
12	125	8-2-80	96	83	86.5	13	0	0	16	80	
12	120	8-2-88	89	72	80.9	17	0	0	0	72	

Table A-1 (cont.)

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est hatch number	Comments
13	126	15-4-3	311	302	97.1	2	7	2.2	7	302	
13	141	15-4-4	318	228	71.7	21	69	21.7	122	196	Aban[d]
13	125	15-4-5	303	288	95.0	15	0	0	108	195	
13	124	15-4-6	257	250	97.3	6	1	0.4	63	194	
13	125	15-4-7	220	208	94.6	9	3	1.4	42	178	
13	138	15-4-13	506	457	90.3	36	13	2.6	121	385	
13	125	15-4-22	325	316	97.2	2	7	0.2	7	316	
13	142	15-4-23	389	336	86.4	37	16	4.1	84	305	
13	119	15-4-25	160	152	95.0	5	3	1.9	13	147	
13	125	15-4-32	325	316	97.2	2	7	2.2	0	316	
13	121	15-4-32a	167	159	95.2	6	2	1.2	29	138	
13	123	15-4-37	225	221	98.2	3	1	0.4	28	197	
13	137	15-4-40	533	512	96.1	15	6	1.1	166	367	
13	119	15-4-50	125	26	20.8	81	18	14.4	3	26	Scat
13	124	15-4-53	253	251	99.2	1	1	0.4	5	248	
13	117	15-4-54	106	98	92.5	8	0	0	6	98	
13	133	15-4-55	221	218	98.6	1	2	0.9	32	189	
13	117	16-1-1	163	149	91.4	14	0	0	6	149	Can
13	143	16-1-2	158	150	94.9	6	2	1.3	20	138	Can
13	126	16-1-3	208	203	97.6	4	1	0.5	48	160	
13	113	16-1-4	116	115	99.1	1	0	0	6	110	Can
13	134	16-1-8	200	197	98.5	0	3	1.5	3	197	
13	125	16-1-9	255	251	98.4	3	1	0.4	5	250	
13	125	16-1-18b	311	300	96.5	7	4	1.3	40	271	
13	129	16-1-19	311	276	88.8	32	3	1.0	115	196	
13	117	16-1-20	161	122	75.8	39	0	0	17	122	

Table A-1 (cont.)

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
13	129	17-5-1	322	312	96.9	8	2	0.6	29	293	
13	125	17-5-6	216	214	99.1	1	1	0.5	9	207	
13	140	17-5-8	637	607	95.3	23	7	1.1	18	607	
13	121	17-5-14a	123	121	98.4	1	1	0.8	15	108	
13	134	17-5-14b	429	426	99.3	2	1	0.2	22	407	
13	136	17-5-15	298	294	98.7	3	1	0.3	26	272	
13	123	17-5-17	288	152	52.8	32	104	36.1	112	152	Aban
13	135	17-5-33	297	297	100	0	0	0	10	287	
13	123	17-5-34	209	189	90.4	20	0	0	36	173	Can
13	147	17-5-37	178	154	86.5	20	4	2.2	19	154	Aban
13	L	17-5-38	125	0	0	0	125	100	0	0	
13	137	17-5-41	495	474	95.8	16	5	1.0	113	382	
13	139	17-5-44	173	171	98.8	2	0	0	9	164	
13	126	17-5-45	265	259	97.7	6	0	0	33	232	
13	123	17-5-49	227	208	91.6	13	6	2.6	53	174	
11	165	22-7-5	282	253	89.7	21	8	2.8	33	249	
11	167	22-7-10a	209	35	16.8	143	31	14.8	184	25	
11	155	22-7-14	6	0	0	4	2	33.3	1	0	Can
11	172	22-7-15	399	368	92.2	28	3	0.8	3	368	Hatch
11	172	22-7-17	229	217	94.8	12	0	0	0	217	Hatch
11	172	22-7-41	338	75	22.2	175	88	26	338	0	Scat
11	154	22-7-43	189	180	95.2	5	4	2.1	21	168	Can
11	165	22-7-54	368	273	74.2	67	28	7.6	203	165	

Table A-1 (cont.)

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
12	118	24-3-3a	445	419	94.2	26	0	0	103	342	
12	119	24-3-4a	367	360	98.1	4	3	0.8	30	337	
12	L	24-3-4b	309	248	80.3	35	26	8.4	7	248	Can
12	116	24-3-14	430	396	92.1	25	9	2.1	39	391	
12	112	24-3-18	77	77	100	0	0	0	3	74	Can, scat
12	125	24-3-23a	279	278	99.6	1	0	0	1	278	
12	125	24-3-23b	158	157	99.4	1	0	0	1	157	
12	121	24-3-25	131	129	98.5	0	2	1.5	16	115	
12	113	24-3-30	304	275	90.5	26	3	1.0	88	216	
12	120	24-3-36	168	167	99.4	1	0	0	10	158	
12	120	24-3-43	309	308	99.7	1	0	0	7	302	Can
12	110	24-3-45	192	181	94.3	11	0	0	0	181	
12	118	24-3-46	309	248	80.3	35	26	8.4	53	248	
12	119	24-3-50	298	294	98.7	1	3	1.0	32	266	
12	111	24-3-54	253	241	95.3	10	2	0.8	31	222	Can
12	103	24-3-56a	67	35	52.2	11	21	31.3	28	35	Can
12	103	24-3-56b	152	152	100	0	0	0	0	152	Hatch
12	103	24-3-57	82	38	46.3	20	24	29.3	12	38	Aban

[a] Can, male eating developing embryos.
[b] Hatch, embryos hatched into larvae.
[c] Scat, brood scattered throughout tube
[d] Aban, male abandoned the brood.
[e] L, original data sheet lost.

Table A-2. Experimental data from Neanthes arenaceodentata mated pairs that were exposed to 0.15 mGy/h from an external gamma-radiation source. The number of days from spawn to hatch and from hatch to spawn as well as the estimated hatch number are provided.

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
5	126	1-2-15	139	139	100	0	0	0	0	139	Hatch[a]
5	159	1-2-18	294	170	57.8	48	54	18.4	94	192	
5	146	1-2-35	286	192	67.1	47	47	16.4	171	115	
5	129	1-2-39	227	209	92.1	11	7	3.1	72	155	
5	146	1-2-40	182	143	78.6	28	11	6.0	109	73	
5	136	1-2-46	231	169	73.2	33	29	12.6	104	127	Can
5	140	1-2-50	154	77	50.0	43	34	22.1	77	77	
10	143	5-1-2	209	161	77.0	20	28	13.4	121	88	
10	109	5-1-3a	241	239	99.2	2	0	0	2	239	Can[b]
10	122	5-1-4	58	32	55.2	15	11	19.0	8	32	Can
10	144	5-1-11	373	270	72.4	68	35	9.4	177	196	
10	114	5-1-12	281	276	98.2	2	3	1.1	7	274	Hatch
10	137	5-1-16	193	163	84.5	22	8	4.2	40	153	
10	108	25-3-1	132	129	97.7	3	0	0	6	126	Hatch
10	121	25-3-2	133	133	100	0	0	0	0	133	Hatch
10	108	25-3-4	209	209	100	0	0	0	0	209	Hatch
10	110	25-3-6	0	0	0	0	0	0	0	0	Can
10	108	25-3-11	163	163	100	0	0	0	0	163	Hatch
10	108	25-3-19	148	148	100	0	0	0	0	148	Hatch
10	108	25-3-23	121	110	90.9	6	5	4.1	7	110	Can
10	110	25-3-24	208	206	99.0	2	0	0	12	196	
10	108	25-3-26	169	154	91.1	0	15	8.9	5	154	
10	103	25-3-29	322	319	99.1	0	3	0.9	6	316	
10	108	25-3-34a	162	159	98.1	3	0	0	13	149	
10	108	25-3-39	160	158	98.8	2	0	0	0	158	Hatch
10	131	25-3-42a	139	118	84.9	14	7	5.0	46	93	
10	110	25-3-42b	169	167	98.8	2	0	0	15	154	
10	108	25-3-43	174	174	100	0	0	0	0	174	Hatch

Table A-2. (cont.)

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
10	108	25-3-45	194	194	100	0	0	0	4	190	
10	109	25-3-47	232	209	90.1	22	1	0.4	26	206	Can
10	132	25-3-48	221	183	82.8	28	10	4.5	138	83	Can
10	110	26-4-3	231	224	97.0	6	1	0.4	13	218	Can
10	114	26-4-5	132	127	96.2	2	3	2.3	19	113	
10	119	26-4-7a	192	181	94.3	6	5	2.6	57	135	
10	133	26-4-7b	178	128	71.9	39	11	6.2	65	113	
10	108	26-4-10	227	227	100	0	0	0	0	227	Hatch
10	129	26-4-13	289	243	84.1	24	22	7.6	77	212	
10	125	26-4-18	315	287	91.1	23	5	1.6	164	151	
10	108	26-4-20a	152	107	70.4	45	0	0	11	107	Can
10	125	26-4-23a	392	17	4.3	169	206	52.6	256	17	
10	125	26-4-23b	83	27	32.5	12	44	53.0	80	3	
10	107	26-4-27	181	180	99.4	0	1	0.6	1	180	Hatch
10	112	26-4-30	21	21	100	0	0	0	0	21	Hatch
10	125	26-4-33	271	253	93.4	16	2	0.7	140	131	Can
10	151	26-4-34b	234	75	32.1	72	87	37.2	80	75	
10	128	26-4-35	187	156	83.4	15	16	8.6	2	156	
10	101	26-4-38a	304	303	99.7	0	1	0.3	12	292	
10	108	26-4-40	190	190	100	0	0	0	0	190	Hatch
12	128	27-5-1	322	258	80.1	52	12	3.7	88	234	
12	105	27-5-3	203	198	97.5	2	3	1.5	5	198	Hatch
12	107	27-5-4	185	185	100	0	0	0	23	162	Can
12	105	27-5-7	116	116	100	0	0	0	0	116	Hatch
12	118	27-5-9	405	331	81.7	52	22	5.4	363	42	
12	110	27-5-13	222	210	94.6	10	2	0.9	49	173	Can
12	102	27-5-14	207	185	89.4	22	0	0	5	185	
12	100	27-5-16	182	174	95.6	7	1	0.5	13	169	Hatch
12	107	27-5-20	96	96	100	0	0	0	0	96	Hatch

Table A-2 (cont.)

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
12	146	29-7-2b	326	302	92.6	16	8	2.5	97	229	
12	112	29-7-3	99	98	99.0	1	0	0	4	95	Hatch
12	131	29-7-5	197	163	82.7	11	23	11.7	51	146	
12	153	29-7-6	494	384	77.7	36	74	15	115	379	
12	122	29-7-7b	231	83	35.9	117	31	13.4	168	63	Can
12	126	29-7-8	309	309	100	0	0	0	0	309	Hatch
12	122	29-7-9	125	105	84.0	13	7	5.6	82	43	
12	126	29-7-10	127	119	93.7	7	1	0.8	46	81	Can
12	142	29-7-11a	226	122	54.0	82	22	9.7	143	83	
12	170	29-7-12b	140	140	100	0	0	0	0	140	Hatch
12	139	29-7-14	507	403	79.5	47	57	11.2	299	208	
12	107	29-7-16	227	226	99.6	0	1	0.4	62	165	
12	122	29-7-17	230	151	65.7	71	8	3.5	165	65	Can
12	112	29-7-19	127	127	100	0	0	0	0	127	Hatch
12	138	29-7-25	256	215	84.0	32	9	3.5	57	199	
12	126	29-7-28b	314	219	69.7	69	26	8.2	223	91	
12	138	29-7-29	217	199	91.7	12	6	2.8	35	182	
12	87	31-8-x	272	272	100	0	0	0	0	272	Hatch
12	97	31-8-4a	163	96	58.9	51	16	9.8	x	96	
12	132	31-8-5	278	278	100	0	0	0	0	278	Hatch
12	105	31-8-7	201	109	54.2	79	13	6.5	98	103	Can
12	128	31-8-9a	306	104	34.0	101	101	33.0	306	0	
12	131	31-8-9b	229	201	87.8	15	13	5.7	0	201	Hatch
12	136	31-8-13a	71	4	5.6	21	46	64.8	71	0	Can
12	131	31-8-14	158	158	100	0	0	0	0	158	Hatch
12	133	31-8-15	84	11	13.1	39	34	40.5	60	3	
12	131	31-8-27	344	341	99.1	3	0	0	3	341	Hatch

[a] Hatch, embryos hatched into larvae.
[b] Can, male eating developing embryos.

Table A-3. Experimental data from Neanthes arenaceodentata mated pairs that were exposed to 2.1 mGy/h from an external gamma-radiation source. The number of days from spawn to hatch and from hatch to spawn as well as the estimated hatch number are provided.

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
12	115	11-4-1	188	171	91.0	17	0	0	20	168	Can[a]
12	121	11-4-5	116	0	0	103	13	11.2	55	0	
12	127	11-4-8	297	89	30.0	157	51	17.2	90	89	Can
12	119	11-4-11	165	153	92.7	2	10	6.1	48	117	
12	125	11-4-14a	113	43	38.1	57	13	11.5	53	43	Can
12	119	11-4-16	189	175	92.6	4	10	5.3	28	161	
12	123	11-4-17	189	58	30.7	96	35	18.5	78	58	
12	123	11-4-22	201	201	100	0	0	0	8	193	Hatch[b]
12	128	11-4-25	212	104	49.1	17	91	42.9	138	74	
12	151	11-4-33	478	319	66.7	109	50	10.5	177	301	
12	125	11-4-35	62	29	46.8	8	25	40.3	31	29	Can
12	118	11-4-45	220	186	84.5	25	9	4.1	38	182	
12	137	11-4-52	181	100	55.2	34	47	26.0	110	71	Aban[c]
12	125	11-4-53	202	116	57.4	41	45	22.3	86	116	Can
13	114	16-2-3	256	245	95.7	10	1	0.4	11	245	
13	123	16-2-7	329	206	62.6	120	3	0.9	165	164	
13	118	16-2-11a	248	78	31.8	132	38	15.3	127	78	
13	123	16-2-11b	187	151	80.7	21	15	8.0	120	67	Can
13	125	16-2-14	109	62	56.9	18	29	26.6	96	13	
13	118	16-2-21	132	121	92.1	4	7	5.3	61	71	
13	121	16-2-23	171	108	63.2	13	50	29.2	79	92	
13	113	16-2-34	153	134	87.6	18	1	0.6	0	134	
13	121	16-2-39	227	209	92.1	11	7	3.1	72	155	
13	125	16-2-41	336	94	28.0	181	61	18.2	213	94	
13	120	16-2-51	232	208	89.7	7	17	7.3	20	208	
13	119	16-2-52	219	185	84.5	10	24	11	30	185	
13	115	16-2-53	202	182	90.1	18	2	1	20	182	
13	115	16-2-57	205	198	96.6	7	0	0	13	192	Can

Table A-3. (cont.)

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
12	151	20-1-1	251	24	9.6	65	162	64.8	192	24	
12	106	20-1-10	73	72	98.6	0	1	1.4	1	72	Hatch
12	121	20-1-16	210	167	79.5	19	24	11.4	88	122	
12	151	20-1-24	364	5	1.4	246	80	22	217	5	
12	127	20-1-31	180	96	53.3	67	17	9.4	122	0	
13	128	21-3-2	159	30	18.9	49	80	50.3	69	30	
13	113	21-3-5	237	228	96.2	7	2	0.8	24	213	Can
13	119	21-3-6	69	31	44.9	4	34	49.3	55	0	Can
13	114	21-3-8	278	260	93.5	14	4	1.4	6	260	
13	125	21-3-9	320	230	71.9	20	70	21.9	287	0	
13	119	21-3-10	277	161	58.1	22	94	33.9	148	129	
13	119	21-3-13	357	268	75.1	39	50	14.0	242	115	
13	124	21-3-17	322	101	31.4	42	179	55.6	304	0	
13	116	21-3-18	247	247	100	0	0	0	9	238	Hatch
13	134	21-3-19	374	289	77.3	64	21	5.6	238	135	Can
13	116	21-3-20	169	147	87.0	13	9	5.3	30	138	
13	119	21-3-24	289	256	88.6	31	2	0.7	121	168	
13	120	21-3-26	221	109	49.3	106	6	2.7	78	109	
13	142	21-3-27	172	106	61.6	52	14	8.1	103	69	
11	105	23-5-1	111	108	97.3	2	1	0.9	9	102	
11	117	23-5-3	251	129	51.4	25	97	38.6	26	129	
11	111	23-5-12	199	179	89.9	13	7	3.5	61	138	
11	118	23-5-12a	207	98	47.3	47	62	30	145	62	
11	118	23-5-12b	177	81	45.8	59	37	20.9	163	14	
11	120	23-5-13	388	215	55.4	106	67	17.3	259	129	Can
11	119	23-5-19	335	300	89.6	21	14	4.2	301	34	
11	105	23-5-23	133	132	99.2	1	0	0	6	127	

Table A-3. (cont.)

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
12	128	24-6-2	179	170	95.0	1	8	4.5	56	123	Hatch
12	99	24-6-5	158	153	96.8	5	0	0	0	153	
12	108	24-6-8	259	239	92.3	2	18	7	103	156	Can
12	109	24-6-13	285	84	29.5	81	120	42.1	266	19	
12	100	24-6-17	188	155	82.4	33	0	0	17	155	Can
12	101	24-6-27	268	266	99.3	2	0	0	33	235	
12	111	24-6-28a	167	99	59.3	56	12	7.2	90	77	
12	106	24-6-35a	252	229	90.9	13	10	4	31	221	
12	114	24-6-36	302	221	73.2	52	29	9.6	209	93	Can
12	109	24-6-37	282	232	82.3	39	11	3.9	174	108	
12	105	24-6-39	187	168	89.9	8	11	5.9	45	142	Hatch
12	101	24-6-43	221	195	88.2	26	0	0	22	195	
12	99	24-6-47	139	138	99.3	0	1	0.7	5	134	Hatch
13	176	27-8-1	152	150	98.7	2	0	0	1	150	Hatch
13	168	27-8-2	122	38	31.2	57	27	22.1	93	29	
13	199	27-8-3	388	337	86.9	50	1	0.3	72	316	
13	182	27-8-4	508	114	22.4	308	86	16.9	271	114	Scat[d]
13	174	27-8-6	282	282	0	0	0	0	0	282	Hatch
13	168	27-8-7	188	188	100	0	0	0	0	188	Hatch
13	192	27-8-20	38	20	52.6	14	4	10.5	34	4	Can
13	180	27-8-21	315	315	100	0	0	0	315	0	Scat
13	173	27-8-22	249	245	98.4	0	4	1.6	0	245	Hatch
13	174	27-8-23	267	245	91.8	17	5	1.9	7	245	Hatch
13	173	27-8-24	249	247	99.2	1	1	0.4	0	247	Hatch
13	166	27-8-25	262	160	61.1	45	57	21.8	24	150	

[a] Can, male eating developing embryos.
[b] Hatch, embryos hatched into larvae.
[c] Aban, male abandoned the brood.
[d] Scat, brood scattered in the tube.

Table A-4. Experimental data from Neanthes arenaceodentata mated pairs that were exposed to 17 mGy/h from an external gamma-radiation source. The number of days from spawn to hatch and from hatch to spawn as well as the estimated hatch number are provided.

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
9	128	4-1-8	278	0	0	0	278	100	270	0	Can[a]
9	139	4-1-14	190	98	51.6	56	36	19.0	183	7	Aban[b]
9	137	4-1-15	74	0	0	6	68	91.9	74	0	Can
9	130	4-1-16	190	63	33.2	60	67	35.3	190	0	Can
9	127	4-1-17	397	87	9.1	144	166	41.8	315	82	
9	140	4-1-21	203	3	1.5	130	70	34.5	203	0	Scat[c]
9	131	4-1-22	109	34	31.2	28	47	43.1	103	6	Can
9	137	4-1-23	74	2	2.7	39	33	44.6	74	0	Can
9	140	4-1-33	186	38	20.4	106	42	22.6	186	0	Can
9	120	4-1-35	3	1	33.3	1	1	33.3	2	1	Can
15	125	10-4-2	107	82	76.6	12	13	12.2	67	40	Can
15	133	10-4-3	147	19	12.9	73	55	37.4	147	0	Can
15	136	10-4-4	109	19	17.4	52	38	34.9	107	2	Scat
15	142	10-4-7	132	0	0	10	122	92.4	132	0	Can
15	121	10-4-12	372	190	51.1	50	132	35.5	281	91	Can
15	124	10-4-14	261	49	18.7	209	3	1.2	257	4	Scat
15	135	10-4-18a	261	7	2.7	69	185	70.9	260	1	Can
15	139	10-4-19	85	37	43.5	27	21	24.7	85	0	Can
15	123	10-4-20	523	55	10.5	215	253	48.4	523	0	Can
15	116	10-4-31	161	138	85.7	19	4	2.5	44	117	Can
15	131	10-4-32	91	6	6.6	66	19	20.9	91	0	Can
15	125	10-4-35	186	21	11.3	73	92	49.5	186	0	Can
15	125	10-4-40	349	48	13.8	192	109	31.2	333	16	Can

Table A-4 (cont.)

P_1 Spawn to F_1 hatch (days)	F_1 Hatch to F_1 spawn (days)	ID	Spawn number	Live number	Live (%)	Dying number	Dead number	Dead (%)	Abnormal number	Est. hatch number	Comments
11	141	11-2-2	376	54	14.4	237	85	22.6	210	54	Can
11	159	11-2-13	313	5	1.6	148	160	51.1	313	0	Can
11	139	11-2-15	230	19	8.3	137	74	32.2	214	16	Can
11	143	11-2-17	185	29	15.7	122	34	18.4	175	10	Can
11	137	11-2-24	199	128	64.3	39	32	16.1	187	12	Can
11	134	11-2-26	182	88	48.4	84	10	5.5	164	18	Can
11	134	11-2-27	132	33	25.0	82	17	12.9	90	33	Can
11	145	11-2-29	187	18	9.6	63	106	56.7	187	0	Can
11	142	11-2-30	308	171	55.5	99	38	12.3	285	23	Can
11	142	11-2-39	549	304	55.4	81	164	29.9	547	2	Can
11	147	11-2-40	168	19	11.3	70	79	47	168	0	

[a] Can, male eating developing embryos.
[b] Aban, male abandoned the brood.
[c] Scat, brood scattered in the tube.

Table A-4. (cont.)

All of these females resorbed their oocytes and then died.

ID	Comments
4-1-2	
4-1-4	
4-1-7	
4-1-9	
4-1-18	
4-1-20	
4-1-26	
4-1-27	
4-1-30	
4-1-31	
4-1-32a	
4-1-36	
10-4-1	
10-4-8	
10-4-9	
10-4-13	
10-4-22	
10-4-23	
10-4-30	
10-4-37	
11-2-3	
11-2-7	
11-2-32	
11-2-36	
11-2-38	