To the Graduate Council:

I am submitting herewith a thesis written by Edward A. Bardill entitled "The Effects of Acute Ionizing Radiation on Selected Life Stages of the Calanoid Copepod Diaptomus clavipes Schacht." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Radiation Biology.

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We have read this thesis and recommend its acceptance:

Accepted for the Council:

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Graduate Studies and Research

THE EFFECTS OF ACUTE IONIZING RADIATION ON SELECTED LIFE STAGES OF THE CALANOID COPEPOD *DIAPTOMUS CLAVIPES* SCHACHT

A Thesis

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Degree

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ABSTRACT

The purpose of this investigation was to determine the effects of Cobalt-60 gamma rays on selected life stages of the calanoid copepod Diaptomus clavipes Schacht following acute doses of ionizing radiation ranging from 0 to 100 kilorads.

The study was initiated by establishing a stock culture of adult males and females of approximately the same age in order to obtain the selected life stages necessary for irradiation. The acute ionizing radiation used in the study was administered by means of two Cobalt-60 sources with dose rates of 7000 and 201 rads per minute. The latter source was utilized to deliver the low dose levels (0.1 and 0.25 kilorads) to nauplii. Observations were made daily to determine the effects of acute irradiation on the survival and continued development of each life stage.

A significant decrease in hatching success with increasing dose at levels $\geq$ 1.0 kilorads was observed when all clutches were compared as well as when a comparison of intact and dropped clutches was made. The irradiation of nauplii resulted in a decrease in the percentage reaching the copepodid stage as well as a decrease in survival with increasing dose at levels $\geq$ 0.5 kilorads. The percentage of copepodids reaching adulthood was significantly reduced at levels $\geq$ 1.0 kilorads while the mean survivorship decreased with increasing dose at levels $\geq$ 5.0 kilorads. The mean survivorship of adults decreased significantly at dose levels $\geq$ 1.0 kilorads.

A comparison of the results to those in the literature (Auerbach et al., 1974) suggest that calanoid copepods may be more sensitive than

cladocerans to acute ionizing radiation. An unusual finding was the suggested greater radiosensitivity of nauplii than the embryos tested at dose levels $\leq$ 1.0 kilorad. Several explanations for the more sensitive response of the naupliar stage were discussed:

1. A greater metabolic activity of the nauplii during the first 24 hours of development.

2. A later, less sensitive embryonic stage was irradiated.

Suggestions for future studies include comparative studies of the nauplii and embryos as to sensitivity to ionizing radiation and the effects of chronic irradiation on calanoid copepods.

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CHAPTER I

INTRODUCTION

A. The Problem

The depletion of our natural resources for the production of energy to meet the increasing demands of our population has resulted in the increased construction of nuclear power stations during the last decade. The effects of the radioactive effluents released from these plants on aquatic life is one of the primary considerations used in evaluating the impact on the aquatic ecosystem (Blaylock, 1972). Although the aquatic organisms living in the vicinity of nuclear power stations will be exposed to extremely low levels of radioactivity, accidents could occur which would result in damaging concentrations of radionuclides. In preparation for these contingencies, more information on the sensitivity of the life stages of aquatic organisms is necessary. Information on the radiosensitivity of food chain organisms such as zooplankton which occupy the lower trophic levels of aquatic ecosystems is especially lacking and is required to fully assess the possible ecological effects.

B. Previous Studies

Zooplankton play an important role in aquatic ecosystems of lakes and ponds and serve as an important source of food in grazing food chains. Although chronic radiation studies (Marshall, 1962, 1966, and 1967) and acute radiation studies (Auerbach et al., 1973) have been conducted on Daphnia pulex and Daphnia magna, parthenogenetically reproducing cladocerans, no data have been available on the radiosensitivity of a

species of sexually reproducing zooplankton until recently. A preliminary study (Gehrs et al., 1975) dealing with acute radiation effects on the sensitivity of embryonic and adult female Diaptomus clavipes Schacht, a sexually reproducing calanoid copepod, has suggested that calanoid copepods may be more sensitive than cladocerans to acute irradiation. More information on the radiosensitivity of calanoids is necessary to determine if calanoids are more sensitive than cladocerans and to determine if that sensitivity is differential in regard to the life stages.

C. Purpose and Objectives

The purpose of the present study was to obtain information on the radiosensitivity of selected developmental stages (embryo, naupliar, copepodite, and adult female) of D. clavipes Schacht. Acute ionizing radiation was chosen to investigate the radiobiological response of the different life stages, since there is virtually no information and a baseline must be established before chronic radiation studies can be initiated. The objectives of the present study were as follows:

1. To determine the relative sensitivity of the different developmental stages of D. clavipes to acute ionizing radiation.

2. To compare the radiosensitivity of D. clavipes to Daphnia magna.

3. To compare the results observed in the present study with that of the previous study involving D. clavipes (Gehrs et al., 1975).

A similar study (Holton, 1971) previously examined the effects of acute gamma irradiation (0 to 3 kilorad) on a population of Artemia

(Brine Shrimp) composed of the different developing stages from the seventh instar to mature adults. Artemia is not only a member of the same class, Crustacea, as the calanoid but also is similar in its development. The eggs are carried by the female for one to several days and hatch into a typical nauplius. This study, however, only examined the effects of irradiation on the reproductive ability of Artemia and not the relative sensitivity of the life stages. Until now, no study has dealt with the effects of acute ionizing radiation on different life stages of a calanoid copepod.

CHAPTER II

METHOD AND PROCEDURE

A. The Organism

The genus Diaptomus is a member of the suborder Calanoida, Order Copepoda, Class Crustacea of the Phylum Arthropoda (Hutchinson, 1967). Diaptomus clavipes as well as other members of the genus Diaptomus play an important role in the ecosystems of lakes and ponds. Their important position as an intermediate link in the food chains of ponds and lakes can be a factor in determining species and population size of fish (Barnes, 1968). The organisms used in the present study were from a stock culture maintained at Oak Ridge National Laboratory which was obtained from a Diaptomus clavipes population that inhabits a pond in Cleveland County, Oklahoma (Gehrs, 1974).

B. Life History

The typical life history of D. clavipes consists of the egg, six naupliar stages, five copepodid stages and, finally, the adult (Pennak, 1953). The egg (Figure 1) hatches into a small, compact larva called a Nauplius I (Figure 2) which soon molts to the Nauplius-II stage. In a similar manner, four additional naupliar stages occur until the Nauplius VI stage is reached. In the next molt, there is not only an increase in size but also a morphological transformation to a basically adult form. This is the Copepodid I stage (Figure 3) and is followed by five similar stages with the Copepodid VI being the sexually mature adult (Figure 4). The time necessary for a complete life cycle is highly variable depending

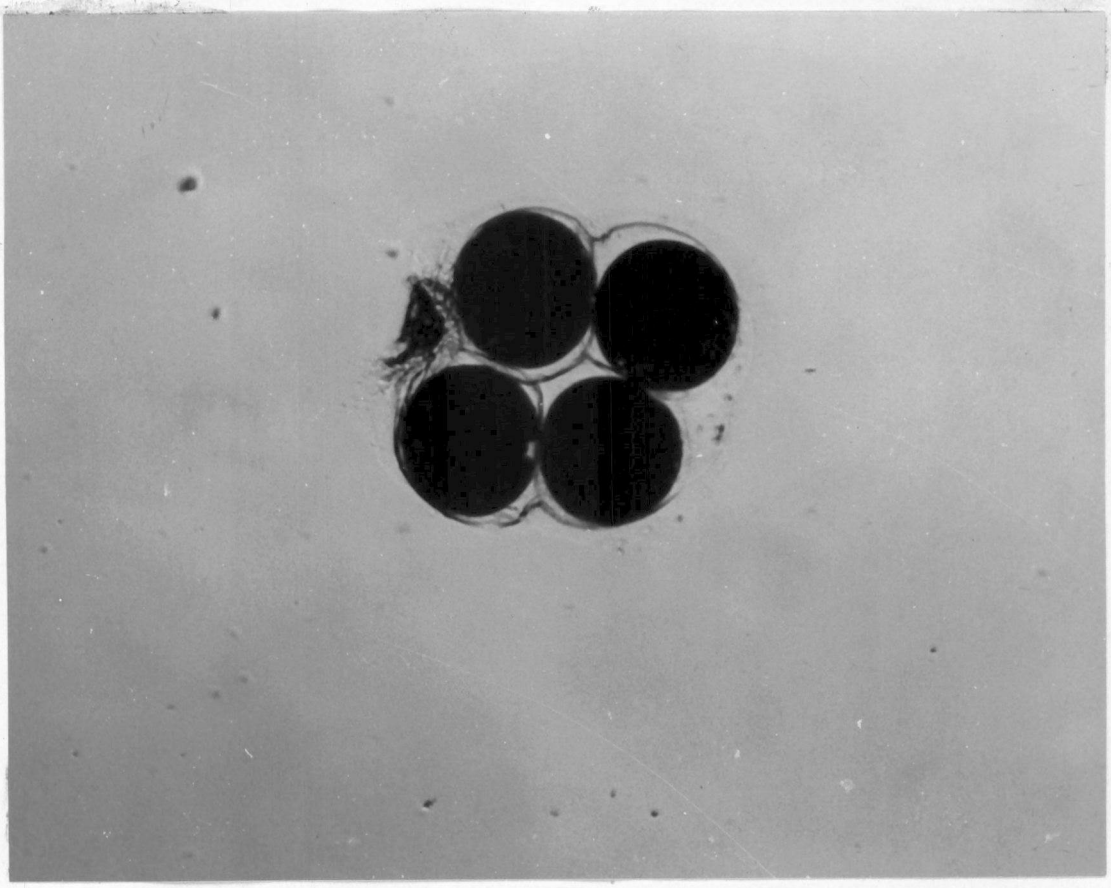

Figure 1. The Embryonic Stage of D. clavipes. x 150.



Figure 2. A Naupliar Stage of D. clavipes. x 400.

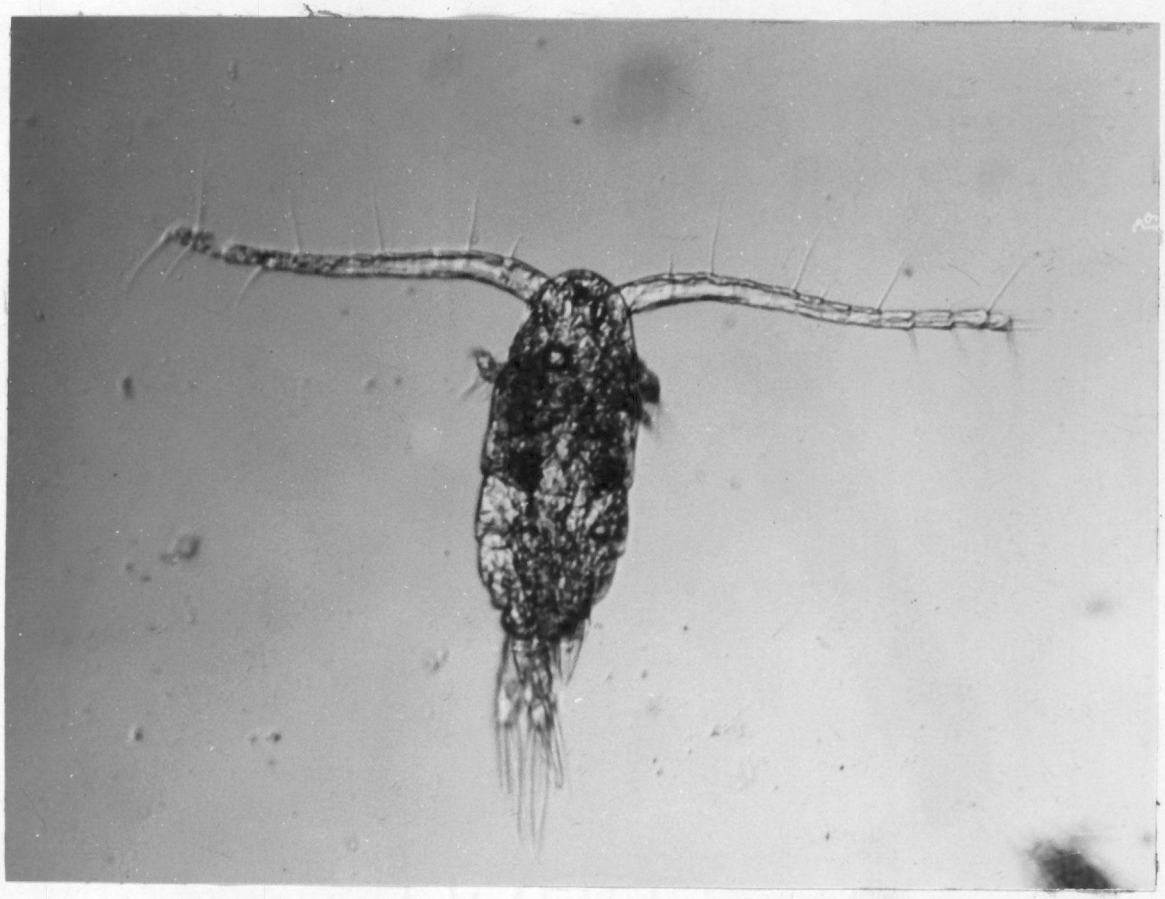

Figure 3. A Copepodid Stage of D. clavipes. x 150.

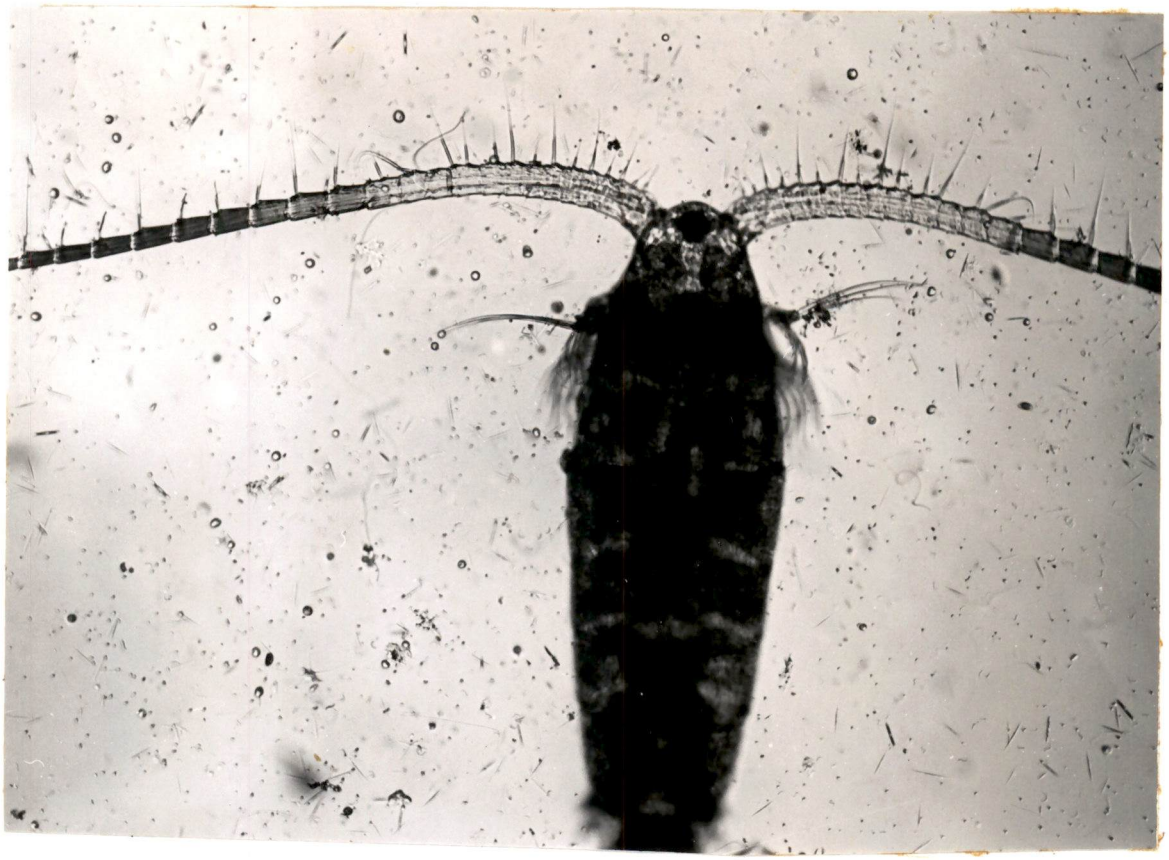

Figure 4. The Adult Female Stage of D. clavipes. x 70.

on species and environmental conditions. Diaptomus clavipes requires 28 days at 15°C (Gehrs and Robertson, 1975), D. reighardi requires 23 days at 21°C (Carter, 1974), while D. ashlandi at 22°C is monocyclic requiring the entire year to complete its life cycle (Comita, 1959).

C. Culture Method

The containers used for stock cultures were ten liter aquaria and selected life stages were maintained in 100 ml glass beakers which corresponds to the recommended containers suggested in a recent study (Robertson et al., 1974). The beakers not only afforded easy arrangement in an environmental chamber, but also facilitated easy observation of the life stages with minimal disturbance of the animals.

The culture medium selected was filtered spring water (See Appendix A) which is more acceptable than conditioned tap water or distilled water (Hardin, 1972). The beakers were filled to a volume of 80 ml which was maintained daily by using watch glasses as covers to minimize evaporation and occasional additions of spring water when the level dropped below 80 ml. The culture media were changed at ten day intervals and on some occasions earlier, due to algae growth.

The beakers and aquaria were kept at a temperature of 21 ± 2°C and a light cycle of 12 hours on and 12 hours off. The temperature and light conditions chosen correspond to those suggested by a previous study which showed that hatch success was maximum at a temperature of 21°C and that a temperature within a few degrees of 25°C would be sufficient for culture conditions (Robertson et al., 1974).

The food medium used was an aqueous mixture of Purina Trout Chow (See Appendix B) prepared by blending 20 grams with 450 ml of distilled water for five minutes in a blender. The mixture was subsequently filtered and the resulting solution kept refrigerated in a stoppered bottle to minimize bacterial growth (Robertson et al., 1974). The advantage of this food medium is the ease with which a reproducible composition can be prepared. The quantity of food medium administered to each beaker was 0.2 ml at two day intervals while aquaria received 0.2 ml per 100 ml of culture medium. Feeding schedules were altered on occasions to prevent accumulation of food which was evidenced by extreme cloudiness of the culture media.

To obtain the different life stages of a known age, it was necessary to have a stock culture of adults of approximately the same age. This was accomplished by removing a total of 70 ovigerous females from a stock laboratory culture and distributing them into seven groups of ten individuals. Each group was then placed into a one liter beaker containing 850 ml of spring water. Food was added at the prescribed intervals and observations were made daily for hatching of eggs. After the eggs hatched, the females were removed and the nauplii were transferred to an aquarium filled with spring water, fed daily, and allowed to develop into adults. When ovigerous females were observed in the aquarium, attainment of CVI instar (Adult) was determined. The aquarium was then emptied by gradually filtering the contents through #20 mesh netting. Additional adult females and males were required to complete the study and the procedure was repeated with another 40 ovigerous females.

Adult males and females were randomly distributed into groups of five males and five females and transferred to each of 15 one liter beakers containing spring water. Observations were made daily to detect egg clutch formation.

D. Irradiation Method

The acute ionizing radiation used in the present study consisted of gamma rays (1.17 and 1.33 MeV) from a Cobalt-60 source with a half-life of 5.26 years and a current dose rate of approximately 7000 rad per minute determined by calculating the activity of the source at the present time. Based on a recent study (Gehrs et al., 1975), five dose levels in the range of 0.1 to 100 kilorad were selected for the life stages (Table I). The animals were irradiated in five 35 ml glass vials containing 25 ml of spring water each, one vial for each dose level and a sixth for the control animals which were subjected to the same handling as the irradiated animals. The total number of replicates for each dose level and controls were as follows: 15 adult females and egg clutches and 30 naupliar and copepodid stages.

The first stages irradiated were the adult female and the egg. Since the female carries the eggs in a brood sac until hatching occurs, it was necessary to irradiate them at the same time with the clutch intact. Since a sufficient number of females with egg clutches is logistically impossible for irradiation at all dose levels in one day, a minimum number of six (one for each dose level and a control) was selected for irradiation purposes. When the minimum number was not available, the females with clutches were placed in separate beakers to obtain nauplii

Table I. Acute Ionizing Radiation Administered to Selected Life Stages of *Diaptomus clavipes*

Dose (Kilorad)	Egg (15 clutches ea.)	Naupliar (30 ea.)	Copepodid (30 ea.)	Adult (30 ea.)
0.1	X			X
0.5	X	X		X
1.0	X	X	X	X
5.0	X	X	X	X
10.0	X	X	X	X
50.0		X	X	
100.0			X	

for irradiation. This procedure was also used to obtain copepodites for irradiation. In addition, control eggs were allowed to develop into nauplii and copepodites to obtain sufficient numbers for irradiation. The time at which a particular life stage was irradiated was kept constant for all groups and dose levels. Adult females were 7 days old. The eggs, nauplii, and copepodites had entered their respective stages within 14 ± 6 hours.

After irradiation, the various stages were transferred to 100 ml beakers containing spring water and returned to the prescribed temperature and light conditions. The irradiated females with clutches were transferred to a watch glass by a capillary tube with an enlarged opening to minimize disturbance of the animal or the egg clutch. A binocular dissecting microscope was used to count the number of eggs present in a clutch. After counting, the females were transferred to beakers and returned to the environmental chamber.

Observations were made daily for mortality, egg hatch, dislodgement of egg clutches or attainment of next transitional stage. When an egg clutch was found to be separated from the female either as a result of dislodgement or hatching of the eggs, the female was transferred to another 100 ml beaker with spring water. In those cases in which the eggs had hatched, 0.5 ml of formalin was added to kill the nauplii except in the case of controls which were used for the naupliar irradiation. The young were then counted and the percent hatch for each clutch was determined by comparing the number of nauplii found to the number of eggs originally carried by the female. Dislodged clutches were allowed to remain in the beaker until hatching occurred. In the few cases in

which females died with the clutch intact, the females were left in the beaker until the eggs hatched or disintegrated.

Owing to an extremely large difference in effects observed between the controls and the first dose level of 0.5 kilorads in the naupliar stages, additional dose levels of 0.1 and 0.25 kilorads were administered to naupliar stages and in addition another group of controls and 0.5 kilorad group were run. The procedures were the same as those mentioned previously with the exception of the source used due to the small dose levels (0.1 and 0.25 kilorads) employed. A Cobalt-60 source (Gammacell 200/ Canada Limited) with a dose rate of 201 rads per minute and an in/ out dose of 14.8 rads was used to administer the required dose levels. The age of the naupliar stages was the same as the previously irradiated nauplii, 14 ± 6 hours.

To identify the particular stage that was irradiated as closely as possible, individuals in excess of the number irradiated were killed with a five percent formalin solution and stored for future reference in glass vials containing a 70 percent alcohol five percent glycerin mixture with several drops of buffered formalin added (Gehrs, 1973). A binocular microscope was used to examine the eggs in an attempt to identify the stage of development and to determine when the copepodites had reached the adult stage by examining its fifth pair of swimming legs (Czaika, 1968).

CHAPTER III

RESULTS

A. Embryonic Development

The eggs of D. clavipes are carried in a sac on the ventral surface of the posterior section of the female's body and remain in this sac until they hatch into nauplii. The parameters selected for determining the effects of ionizing radiation on the embryonic stages are shown in Table II.

Egg clutches were dropped by females prior to hatching at all dose levels $\geq$ 0.5 kilorads while no clutches were dropped at the 0.1 kilorad dose level or controls. The number of clutches dropped by the females did not increase as the dose level increased. A comparison of the percent of total clutches in which no hatching occurred revealed that the mortality of total clutches increased with increasing dose (dose levels $\geq$ 0.5 kilorad). All clutches in the controls and 0.1 kilorad group had some hatching success while all other dose levels had some clutches which experienced 100 percent mortality.

Statistically, t-tests for equal sample sizes (Sokal and Rohlf, 1969) were used to test for significant differences between all groups. Significant differences ($P. \leq 0.05$) in hatching success between controls and irradiated groups were seen when all clutches were used at all dose levels $\geq$ 0.5 kilorad while no significant difference was seen for the 0.1 kilorad group. Similar results were observed when a comparison was made of the percent hatch of clutches which were carried until hatching occurred. A comparison of the percent hatch in those

Table II. Observed Effects of Acute Ionizing Radiation on Embryonic Diaptomus clavipes

Dose (Kilorad)	Percentage of Clutches		Percentage Hatch ± 95% C. I.			
	Dropped	With No Hatch	All Clutches	Carried Clutches	Dropped Clutches	With Some Hatch
0	0	0	79.43 ± 5.7	79.43 ± 5.7		79.43 ± 5.7
0.1	0	0	71.71 ± 6.7	71.71 ± 6.7		71.71 ± 6.7
0.5	0.6	0.6	*62.71 ± 10.9	*63.02 ± 11.6	58.3 ± 0	67.19 ± 10.9
1.0	53.3	20.0	*42.57 ± 14.8	*65.98 ± 3.2	22.1 ± 17.8	*58.04 ± 14.7
5.0	46.7	40.0	*27.51 ± 14.4	*48.18 ± 15.3	3.9 ± 7.6	*55.75 ± 13.9
10.0	46.7	46.7	*20.91 ± 10.9	*29.11 ± 10.5	6.3 ± 12.4	*39.53 ± 10.9

*$\leq$ 0.05 When Compared to Controls Using t-tests for Equal Sample Sizes.

clutches which were dropped revealed a decrease in percent hatch with increasing dose. In comparing the percent hatch of clutches in which some hatch occurred, a significant decrease in hatch success with increasing dose was seen at dose levels of 1, 5, and 10 kilorad; however, the hatch success of the 0.1 and 0.5 kilorad groups was not significantly lowered from that of the controls. When hatching success in both carried clutches and dropped clutches were compared, a significant difference exists in the 1, 5, and 10 kilorad groups with the dropped clutches significantly below carried clutches.

B. Naupliar Development

The parameters selected for determining the effects of ionizing radiation on the nauplii of D. clavipes are shown in Table III. Nauplii were observed to have reached the Copepodid I stage in all groups which received < 1.0 kilorad while all groups receiving ≥ 1.0 kilorad suffered 100 percent mortality before reaching the copepodid stage. A comparison of the percent of nauplii reaching the copepodid stage at dose levels < 1.0 kilorad showed an increase in the percentage of the successful nauplii with decreasing dose. A similar result was observed when comparing the percent of nauplii which reached Copepodid I and completed their development to the mature adult stage, Copepodid VI. Tests for equality of two percentages (Sokal and Rohlf, 1969, P. 607) resulted in significant differences in the percent of nauplii that reached the copepodid and adult stages for all dose levels in which some nauplii completed development (0.1, 0.25, and 0.5 kilorads). In order to test the significant differences in the amount of time required for the

Table III. Observed Effects of Acute Ionizing Radiation on Naupliar Diaptomus clavipes

Dose (Kilorad)	Percentage			Mean Time (Days)		
	Nauplii Reaching C1	C1 Reaching C6	Total Reaching C6	To C1	To C6	Survival ± 95% C. I.
0	95.6	57.1	53.3	5.9	11.6	26.4 ± 7.6
0.1	*76.7	*39.1	*30.0	5.7	12.8	
0.25	*66.7	*35.0	*23.3	5.5	13.8	
0.5	*21.6	*30.7	*13.3	7.4	13.6	**7.3 ± 1.9
1.0	0	0	0	0	0	**6.6 ± 1.3
5.0	0	0	0	0	0	**5.3 ± 0.9
10.0	0	0	0	0	0	**3.6 ± 0.7
50.0	0	0	0	0	0	**1.8 ± 0.04

*--0.05 When Compared to Controls Using Tests for Equality of Two Percentages.

**--0.05 When Compared to Controls Using t-tests for Equal Sample Sizes.

nauplii to reach the C-I and C-VI stages, as well as survival, t-tests for unequal and equal sample sizes respectively were used. No significant delay in the time to C-I and C-VI was observed for any groups. However, the survivorship of nauplii after irradiation was significantly decreased in the 0.5, 1, 5, 10, and 50 kilorad groups. The percent survival of the 0.1 and 0.25 kilorad groups are omitted because of a malfunction of the temperature control in the environmental chamber causing 100 percent mortality. The survivorship curves of the nauplii for all eight groups are shown in Figure 5. The mean survivorship in all groups ranged from 26.4 to 1.8 days for the control and 50 kilorad groups respectively. After five days, only 7 percent of the controls had perished while the 50 kilorad group had all perished by day four. At day ten, 24 percent of the controls had perished while the 5 and 10 kilorad groups had completely perished. The time necessary for the controls to be reduced 50 percent was 20 days while during the same time period, the 0.5 and 1.0 kilorad groups had suffered 100 percent mortality.

C. Copepodid Development

The control group as well as the 1, 5, and 10 kilorad groups had some copepodids which developed into adults while in the 50 and 100 kilorad groups none survived to adulthood (Table IV). Tests for equality of two percentages showed significant differences in the percent of copepodids reaching the adult stage in the 5 and 10 kilorad groups. Utilizing t-tests for unequal sample sizes, a significant delay in the amount of time for development of copepodids into adults was observed

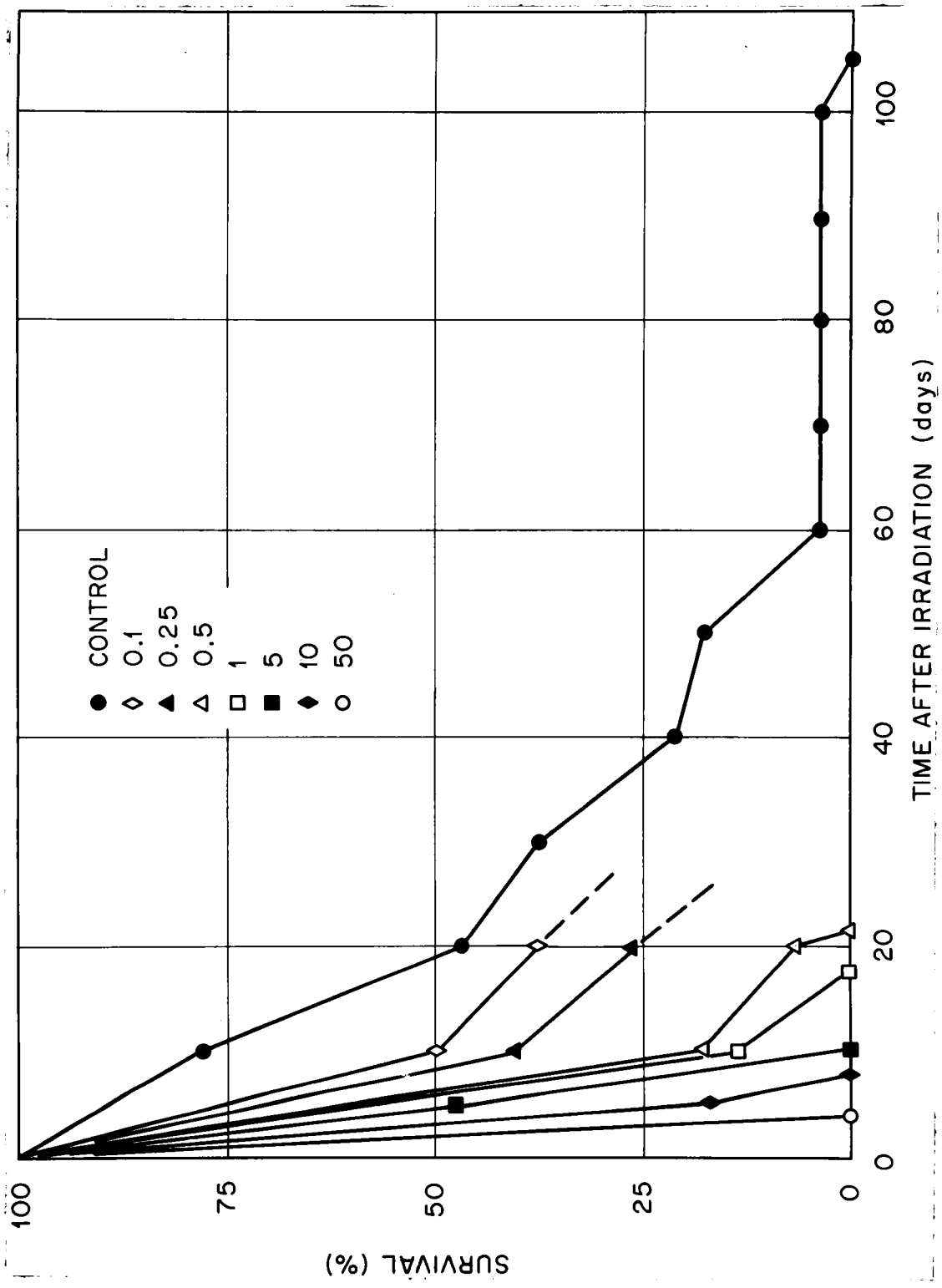

Figure 5. Survivorship curves for D. clavipes nauplii following different doses of acute irradiation.

Table IV. Observed Effects of Acute Ionizing Radiation on Copepodid Diaptomus clavipes

Dose (Kilorad)	# C1 Reaching C6	% C1 Reaching C6	Mean Time (Days) To C6 ± 1 S. E.	Mean Time (Days) Survival ± 95% C. I.
0	16	53.3	11.56 ± 1.3	30.9 ± 10.6
1.0	13	43.3	*15.00 ± 1.8	19.8 ± 7.6
5.0	4	***13.3	*16.50 ± 2.9	**12.0 ± 4.1
10.0	1	*** 3.3	*20.00 ± 0.0	** 6.5 ± 2.6
50.0	0	0	0	** 2.3 ± 0.4
100.0	0	0	0	** 1.7 ± 0.4

*$\leq$ 0.05 When Compared to Controls Using t-tests for Unequal Sample Sizes.

**$\leq$ 0.01 When Compared to Controls Using t-tests for Equal Sample Sizes.

***$\leq$ 0.05 When Compared to Controls Using Tests for Equality of Two Percentages.

for all groups in which some copepodids reached the adult stage (1, 5, and 10 kilorads). The mean survivorship in all groups ranged from 30.9 to 1.7 days for the control and 100 kilorad groups respectively. The survival of copepodids after irradiation was significantly reduced in the 5, 10, 50, and 100 kilorad groups. No significant difference was observed for the 1 kilorad group; however, the malfunction of the environmental chamber previously mentioned also resulted in the mortality of the few remaining control animals (three individuals).

Survivorship curves for all groups of organisms irradiated as copepodids are shown in Figure 6. After five days, the control group as well as the 1, 5 and 10 kilorad groups had well above 60 percent surviving while the 50 and 100 kilorad groups had suffered 100 percent mortality by days three and four respectively. By day 40, the 5 and 10 kilorad groups had already suffered 100 percent mortality while the control and 1 kilorad group were above 20 percent survival.

D. Adult Survivorship

Comparison of the survivorship curves for the adults (Figure 7) following irradiation reveals several interesting observations. By the 10th day, all dose levels had experienced 14-20 percent mortality while no control animals had perished. No mortality occurred in control animals until day 19 by which time approximately 67 and 74 percent each in the 5 and 10 kilorad groups had died. Mean survivorship of individuals in the six groups (Table V) ranged from 47.53 (Control) to 18.87 days (10 kilorad). All treatment groups $\geq$ 1.0 kilorad showed significantly

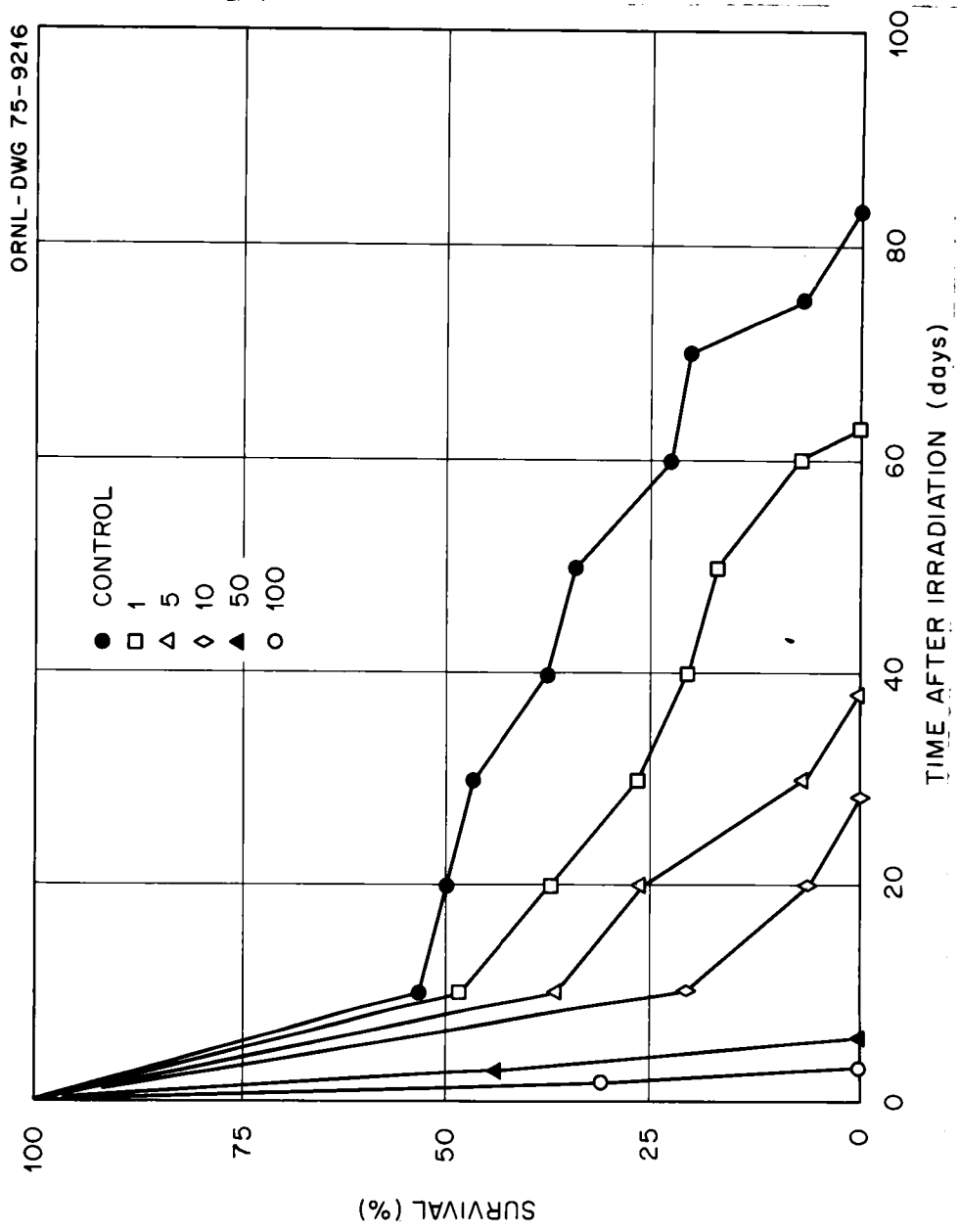

Figure 6. Survivorship curves for D. clavipes copepodites following different doses of acute irradiation.

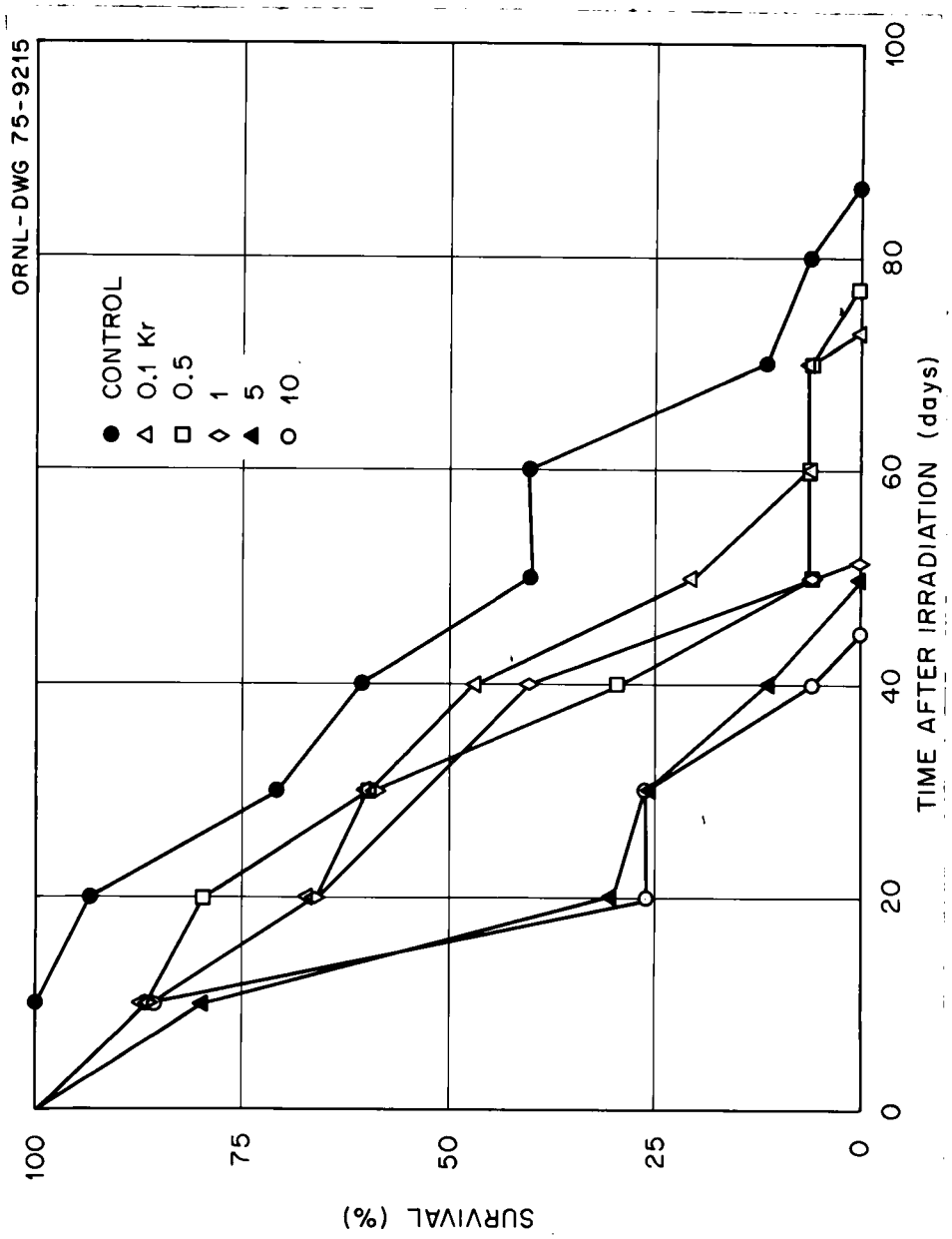

Figure 7. Survivorship curves for adult female D. clavipes following different doses of acute irradiation.

Table V. Mean Survivorship of Adults Following Different Doses of Acute Ionizing Radiation

Dose (Kilorad)	Mean Survivorship ± 95% C. I.	Probability When Compared to Controls
0 (Control)	47.53 ± 10.0	
0.1	33.93 ± 10.1	≥ 0.05
0.5	33.87 ± 9.2	≥ 0.05
1.0	32.26 ± 8.6	≤ 0.05
5.0	19.87 ± 7.3	≤ 0.01
10.0	18.87 ± 6.1	≤ 0.01

lower mean survivorship than the controls ($P \leq 0.05$ for 1 kilorad and $P \leq 0.01$ for 5 and 10 kilorad groups).

CHAPTER IV

DISCUSSION

A. Sensitivity of the Stages

Significant effects of acute irradiation were seen in the adult and copepodid stages at the 1 and 5 kilorad dose levels while in the embryonic and naupliar stages the effects were observed at the 0.5 and 0.1 kilorad dose levels respectively. The most significant finding was the greater sensitivity of the naupliar stage compared to the embryonic stage. In many marine invertebrates and vertebrates, radiotolerance increases with age and is particularly pronounced during embryogenic and early post-embryogenic life stages (Kinne, 1970). Several possibilities might explain the unexpected greater sensitivity of the naupliar stages as compared to the embryonic stages.

The first possibility is that the nauplii are more sensitive owing to a greater metabolic activity during the first 24 hours when they go through several successive molts. It has been established that the radiotolerance of cells is closely correlated with their metabolic state; active cells are more sensitive to ionizing radiation than resting cells or tissues (Kinne, 1970). Dry and incapsulated eggs of the brine shrimp Artemia salina have a very low metabolic rate and are more radiotolerant than those resuming development after water uptake (Rugh and Clugston, 1955).

The second possibility is that the embryonic stages were irradiated during a later stage which was less radiosensitive than a much earlier

stage. Nakanishi and authors (1964) report a marked increase of radiotolerance with increasing age in embryos of Artemia salina following gamma irradiation from a Cobalt-60 source administered at different time intervals up to 15 hours after initiation of development. Kulikov (1966) demonstrated that the radiosensitivity of mollusk embryos decrease substantially from early stages to later stages: LD_{50} of 450R in early blastula stage and a LD_{50} of 2000R at stage of beginning shell formation. Further evidence of increasing tolerance to ionizing radiation with increasing age has been obtained in studies of developing embryos of fish (Bonham and Welander, 1963).

Additional studies in which the embryonic calanoids are irradiated at different time intervals after formation of the eggs will be required to determine if the very early embryonic stages are more sensitive than the stage irradiated in the present study.

B. Comparison of Previous Studies

In a study of acute ionizing radiation on Daphnia magna (Auerbach et al., 1974), results showed significant reductions in total number of young produced and reduced life-span at dose levels $\geq$ 3 kilorads. The results of the present study show that calanoid copepods are more sensitive since embryonic, copepodid, and adult calanoids were affected by doses $\geq$ 1 kilorad and nauplii were affected by all dose levels $\geq$ 0.1 kilorad.

The results of the present study in regard to the effects of acute ionizing radiation on embryonic and adult calanoids agree very well with the findings of Gehrs et al. (1975). The latter study conducted in the

summer of 1974 showed the mean percent hatch of the control, 1 and 10 kilorad groups to be 72.3, 41.3, and 16.5 percent respectively. Table II (P. 16) shows the mean percent hatch for the same dose groups in the former study to be 79.4, 42.6, and 20.9 percent. The adult survivorship in the previous study for the control, 1, and 10 kilorad groups was 49.2, 35.2, and 25.3 days while the results of the present study (Table V, P. 25) show the values for the same dose groups to be 47.5, 32.2, and 18.8 days. In addition, the radiation induced dropping of egg clutches was also observed in both studies.

Hug (1960) observed many instantaneous reactions of invertebrates to ionizing radiations. In three species of snails, the tentacles are retracted and remain so for duration of irradiation. A sea-water snail and a clam also show reflex responses of their tentacles. The threshold for this reflex-like response was from 1.5 to 5 R per second. The dose rate in the present study was well above this level and the dropping of clutches at dose levels $\geq$ 0.5 kilorad may have resulted from a similar reaction. Involvement of the central nervous system of mammals at sub-lethal doses (50R) results in nausea and vomiting (Lebedinsky *et al.*, 1959). Gehrs *et al.* (1975) suggested that if the loss of clutches resulted from a similar response of the central nervous system, an interesting parallel could be drawn between higher organisms and invertebrates. Another finding which was similar to the previous study was the substantial difference in hatching success between the carried and dropped clutches at all dose levels $\geq$ 1.0 kilorad. In the preliminary study (Gehrs *et al.*, 1975), these results suggested the possibility that the female possesses a mechanism which not only assesses the physiological

well being of the eggs but ejects a clutch containing damaged eggs. The findings of the present study provide additional evidence to this theory. The fact that the controls experienced no dropped clutches may be due to more experience in handling of the animals during transfer procedures than when the previous study was conducted.

C. Summary

The results of the present study are important as the first information on the radiosensitivity of the different life stages in the complete life history of a calanoid copepod. The results reinforce the findings of a previous study which suggested that calanoid copepods may be more sensitive than cladocerans to acute irradiation. One of the most significant findings brought out by the present study was the greater sensitivity of the naupliar stage as contrasted to the embryonic stage.

Further study is suggested in certain areas by the results obtained. The results discussed earlier, indicating the more sensitive response of nauplii, are important enough to warrant an intensive comparative study of the embryo and nauplii. The effects of chronic irradiation on the selected life stages of D. clavipes should be examined. This study would not only provide additional information on the radiosensitivity of the different life stages but would also provide for comparisons with previous studies involving chronic irradiation effects on cladocerans.

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APPENDIXES

APPENDIX A

ANALYSIS OF SPRING WATER[a]

Determination	Concentration Measured	Method
Potassium	660 ppb	flame emission
Sodium	430 ppb	atomic absorption
Calcium	26.6 ppm	atomic absorption
Magnesium	13.5 ppm	atomic absorption
Strontium	25 ppb	flame emission
Manganese	<5 ppb	atomic absorption
Iron (II)	<20 ppb	atomic absorption
Iron, total	<5 ppb	atomic absorption
Silver	<300 ppt	spark source mass spec.
Gold	<300 ppt	spark source mass spec.
Barium	81 ppb	flame emission
Cadmium	<100 ppt	atomic absorption
Chromium	<3 ppb	atomic absorption
Copper	<5 ppb	atomic absorption
Mercury	50 ± 10 ppt	flameless atomic absorp.
Lead	<1 ppb	atomic absorption
Zinc	19 ppb	atomic absorption
pH, from spring	6.2	pH meter
pH, after aeration	7.8	pH meter
Total alkalinity	10 ppm	volumetric
Carbonate	<2 ppm	volumetric
Bicarbonate	10 ppm	volumetric
Hydroxide	<2 ppm	volumetric
Sulfate	<1 ppm	spectrophotometric
Phosphate	3 ppb	spectrophotometric
Fluorine	<100 ppb	spectrophotometric
Chlorine	<500 ppb	spectrophotometric
DOC, spring water	0.1 to 0.4 ppm	infrared
DOC, ORNL distilled water	0.08 ppm	infrared
Total nitrogen	<100 ppb	Kjeldahl
Settleable solids	<200 ppb	gravimetric
Total solids	126 ppm	gravimetric
Nonvolatile solids	57 ppm	gravimetric

[a]November 1972 sample from spring water piped into Building 2001, ORNL; analyses performed by members of the Analytical Chemistry Division, ORNL.

APPENDIX B

GUARRANTEED ANALYSIS OF PURINA TROUT CHOW

(SIZE 4) RALSTON PURINA CO.

Crude protein not less than	40.0%
Crude fat not less than	2.5%
Crude fiber not more than	5.5%
Ash not more than	13.0%
Added minerals not more than.	3.0%

INGREDIENTS

Fish meal, soybean meal, ground wheat, corn gluten meal, brewers' dried yeast, ground yellow corn, wheat middlings, dried blood meal, dried whey, dicalcium phosphate, iodized salt, vitamin A supplement, D activated animal sterol, metadione dimethylpyrimidinol bisulfite (source of vitamin K activity), vitamin E supplement, vitamin B12 supplement, ascorbic acid, chlorine chloride, folic acid, pyridoxine lyorochloride, thiamin, niacin, calcium pantothenate, riboflavin supplement, copper sulfate, manganous oxide, zinc oxide, calcium carbonate, cobalt carbonate.

VITA

Edward A. Bardill was born in Harriman, Tennessee on December 20, 1947. He attended elementary schools in that city and was graduated from Harriman High School in 1965. The following September he entered Tennessee Technological University, and in May 1969, he received a Bachelor of Science degree in Biology as well as a commission of Second Lieutenant in the United States Army. In July 1969, he entered upon active duty and attended the Chemical Officer Basic Course at Ft. McClellan, Alabama. Upon graduation in October of that year, he was assigned to U. S. Army Europe in West Germany. At the conclusion of his overseas tour in July 1972, he was once again assigned to Ft. McClellan, Alabama for the purpose of attending the Chemical Officer Advanced Course. He graduated in June 1973 and was selected to attend advanced civilian schooling at a university for the purpose of receiving a Master of Science degree.

He entered the Graduate School at the University of Tennessee in June 1973 and received the Master of Science degree in Radiation Biology in August 1975.

He is married to the former Rita S. Abel of Gunzenhausen, West Germany.