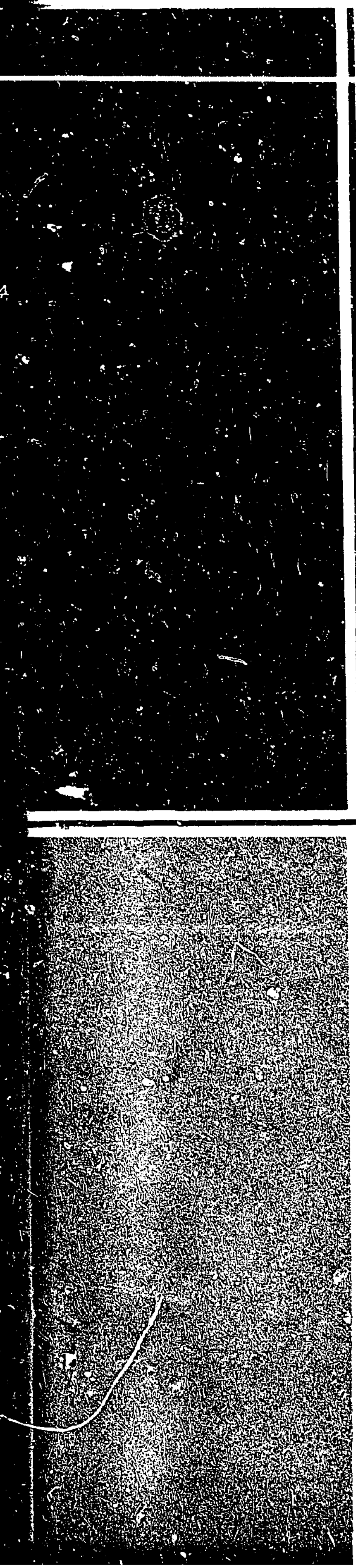

ORNL/TM-5060

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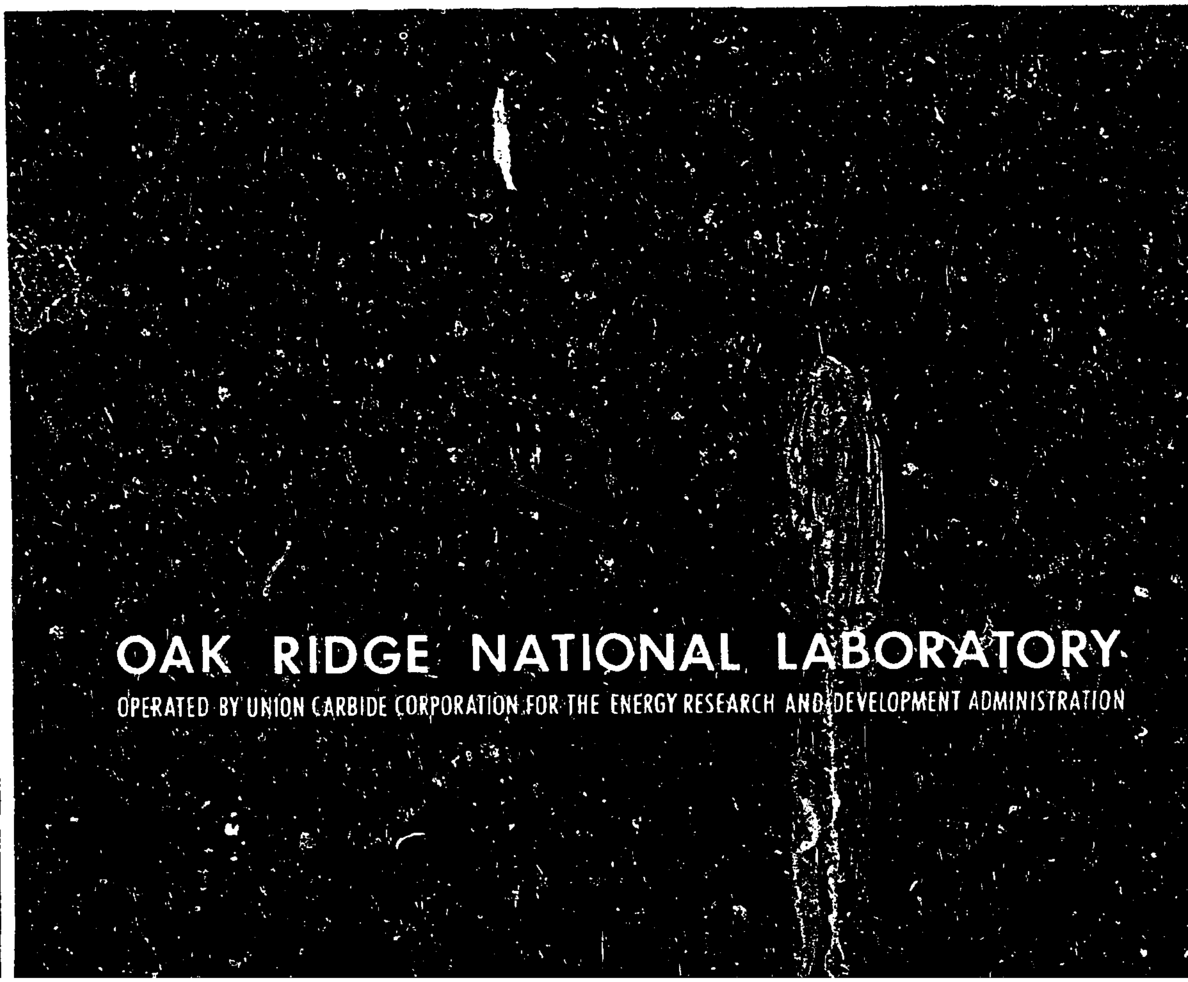

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THE EFFECTS OF ACUTE IONIZING RADIATION ON SELECTED LIFE STAGES OF THE CALANOID COPEPOD DIAPTOMUS CLAVIPES SCHACHT

E. A. Bardill, B. G. Blaylock, C. W. Gehrs, and J. R. Trabalka

Submitted as a thesis by E. A. Bardill to the Graduate Council of the University of Tennessee in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

BARDILL, E. A., B. G. BLAYLOCK, C. W. GEHRS, and J. R. TRABALKA. 1976. The effects of acute ionizing radiation on selected life stages of the calanoid copepod Diaptomus clavipes. ORNL/TM-5060. Oak Ridge National Laboratory, Oak Ridge, Tennessee. pp. 36

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 krad.

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 ^{60}Co sources with dose rates of 7000 and 201 rad/min. 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 was observed at dose levels equal to or greater than 1.0 krad. The irradiation of nauplii resulted in a decrease in the percentage reaching the copepodid stage as well as a decrease in survival with dose at levels equal to or greater than 0.5 krad. The percentage of copepodids reaching the adult stage was significantly reduced at levels equal to or greater than 1.0 krad while the mean survivorship decreased with increasing dose at levels greater than or equal to 5.0 krad. The mean survivorship of adults decreased significantly at dose levels greater than or equal to 1.0 krad.

A comparison of the results to those in the literature suggest that calanoid copepods may be more sensitive than cladocerans to acute ionizing radiation. An unusual finding was the suggested greater radiosensitivity of nauplii stages than that of embryonic stages. Several explanations for the more sensitive response of the naupliar stage were discussed:

(1) the nauplius exhibits a greater metabolic activity during the first 24 hr of development, and

(2) embryos may have irradiated at a later, less sensitive embryonic stage.

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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INTRODUCTION

The depletion of petroleum resources for the production of energy and the increasing demands of society for environmentally clean and economical power has resulted in the increased construction of nuclear power stations during the last decade. The effect of the radioactive effluents released from these plants on aquatic life is one of the considerations used in evaluating their impact on the aquatic ecosystem (Blaylock, 1972). Although 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 the releases of much greater concentrations of radionuclides. To assess the effect of significant releases of radioactivity, more information on the sensitivity of the life stages of aquatic organisms is necessary. Information on the radiosensitivity of important 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.

Zooplankton play an important role in lake and pond ecosystems, serving as an important source of food in grazing food chains. 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. In a related study, Holton (1971) examined the effects of acute gamma irradiation (0 to 3 krad) 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 developmental mode. The eggs are carried by the female for one to several days and are hatched into a typical nauplius. This study only examined the effects of irradiation on the reproductive ability of *Artemia* and not on the relative sensitivity of the life stages. Until recently no data have been available on the radiosensitivity of a species of sexually reproducing zooplankton. In a preliminary study Gehrs *et al.* (1975) suggested that *D. clavipes*, a sexually reproducing calanoid copepod, may be more sensitive to acute irradiation than cladocerans. However, more information on the radiosensitivity of calanoids is necessary to determine if calanoids are more sensitive than cladocerans and to determine if that sensitivity differs significantly among the life stages.

The purpose of the present study was to obtain information on the radiosensitivity of selected developmental stages (embryo, nauplius, copepodide, and adult female) of *D. clavipes* by using acute exposures of ionizing radiation and to establish baseline data for future studies with chronic radiation. 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, and

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

METHODS AND PROCEDURES

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

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 (Fig. 1) hatches into a small, compact larva called a Nauplius I (Fig. 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 an increase in size and also a morphological transformation to the basically adult form. This is the Copepodid I stage (Fig. 3) and is followed by five similar stages, with the Copepodid VI being the sexually mature adult (Fig. 4). The time necessary for a complete life cycle is highly variable depending on species and environmental conditions. Diaptomus clavipes requires 28 days to complete its life cycle (Gehrs and Robertson, 1975), and D. reighardi requires 23 days (Carter, 1974), however D. ashlandi is monocyclic and requires the entire year to complete its life cycle (Comita, 1959).

Ten-liter aquaria were used for stock cultures, and selected life stages were maintained in 100-ml glass beakers (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 was filtered spring water which is more acceptable than conditioned tap water or distilled water (Hardin, 1972). (See Appendix A for analysis of the spring water). The beakers were filled to a volume of 80 ml and maintained at this volume by using watch glasses as covers to minimize evaporation and by occasional additions of spring water. The culture media were changed at ten-day intervals and on some occasions earlier, due to algae growth.

PHOTO 6920-76

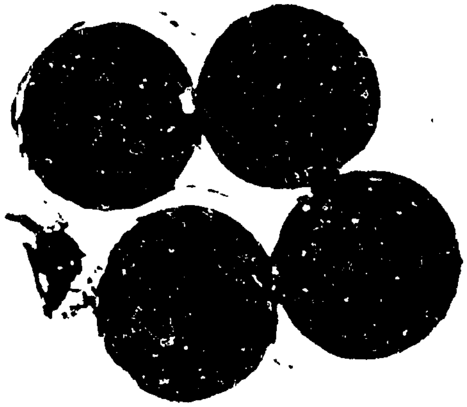

Fig. 1. Embryonic stage of *Diaptomus clavipes* (x 150).

Fig. 2. Naupliar stage of Diaptomus clavipes (x 400).

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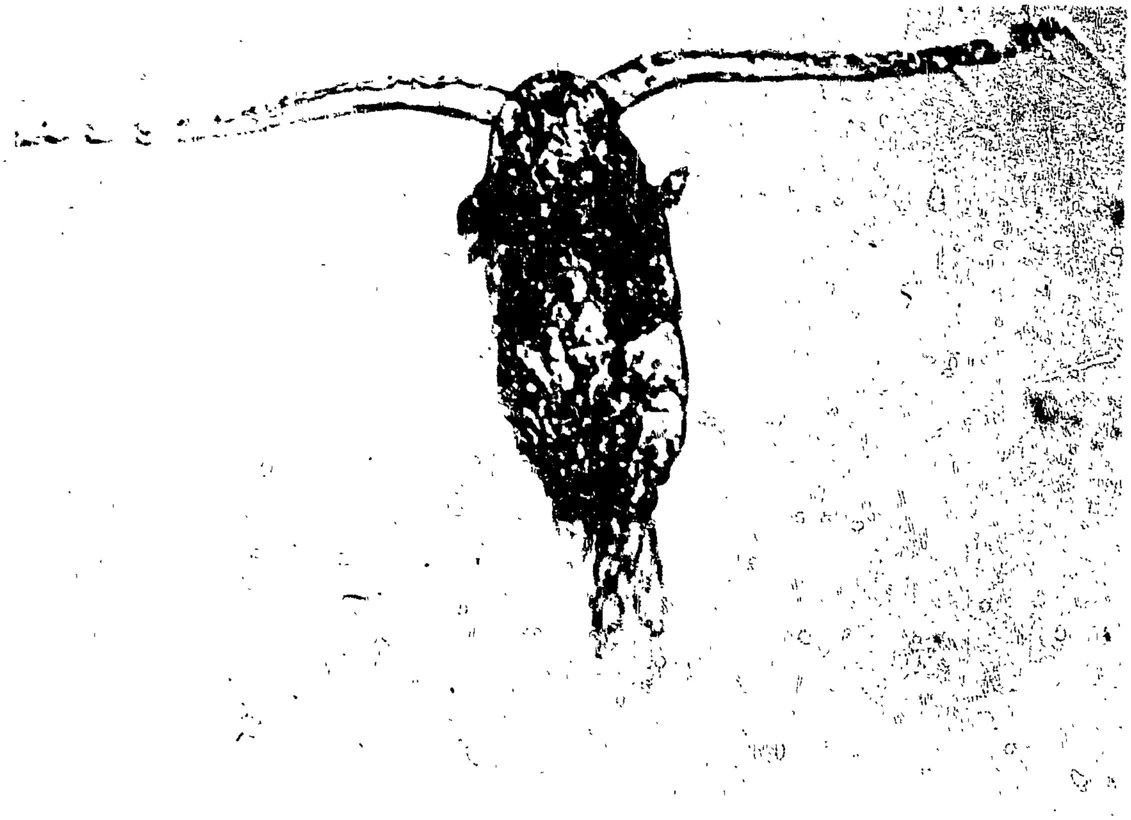

Fig. 3. Copepodid stage of Diaptomus clavipes (x 150).

PHOTO 6921-76

Fig. 4. Adult female stage of <u>Diaptomus</u> <u>clavipes</u> (x 70).

The beakers and aquaria were kept at a temperature of 21 ± 2°C and a photoperiod of 12 hr light and 12 hr dark. These conditions correspond to those suggested by a previous study which showed that hatching success was maximum at a temperature of 21°C and that temperatures within a few degrees of 25°C were adequate for culture conditions (Robertson et al., 1974).

The food medium was an aqueous mixture, prepared by blending 20 grams of Purina Trout Chow (See Appendix B) with 450 ml of distilled water for five minutes. The mixture was filtered and the resulting medium refrigerated in a stoppered bottle to minimize bacterial growth (Robertson et al., 1974). An advantage of this food medium is the ease with which the composition can be reproduced. The quantity of food medium administered to each beaker was 0.2 ml at two day intervals; aquaria received 0.2 ml per 100 ml of water. 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 maintain 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 was made daily for hatching of eggs. After the eggs hatched, the females were removed and the nauplii transferred to an aquarium filled with spring water, fed daily, and allowed to develop into adults. When ovigerous females were observed in the aquarium, adults were collected by gradually filtering the contents through #20 mesh netting.

After collection, groups of five adult males and five adult females were randomly distributed into 15 one-liter beakers and observed daily to detect egg clutch formation. The procedure was repeated with 40 ovigerous females to obtain additional adults.

The acute ionizing radiation used in the present study consisted of gamma rays (1.17 and 1.33 MeV) from a ^{60}Co source with a half-life of 5.26 years and a current dose rate of approximately 7000 rad/min. Based on a recent study (Gehrs et al., 1975), five dose levels in the range of 0.1 to 100 krad were selected for the life stages (Table 1). 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 procedure as the irradiated animals. The total number of replicates for each dose level and controls was 15 adult females with egg clutches and 30 naupliar and copepodid stages.

The first stages irradiated were the adult female and the egg. A sufficient number of females with egg clutches could not be obtained to

Table 1. Acute ionizing radiation administered to selected life stages of *Diaptomus clavipes*

Dose (krad)	Egg[a]	Naupliar[b]	Copepodid[b]	Adult[b]
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	

[a]15 clutches

[b]30 clutches

accomplish irradiation at all dose levels in one day; therefore a minimum of six (one for each dose level and a control) were selected for irradiation purposes. When the minimum was not available, the females with clutches were placed in separate beakers to obtain nauplii for irradiation. This procedure was also used to obtain copepodids for irradiation. In addition, control eggs were allowed to develop into nauplii and copepodids 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. The adult females were 7 days old while the eggs, nauplii, and copepodids had entered their respective stages within 14 ± 6 hr.

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 and the number of eggs in each clutch was determined with the aid of a binocular dissecting microscope.

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 controls which were used for the naupliar irradiation. 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 a few cases females died with the clutch intact; these females were left in the beakers until the eggs hatched or disintegrated.

A large difference in mortality was observed between the controls and the naupliar stages, which had been irradiated with 0.5 krad. Therefore, dose levels of 0.1 and 0.25 krad were administered to naupliar stages and an additional experiment was conducted with another group of controls and a 0.5-krad group. The procedures were the mentioned previously with the exception of the source used. A ^{60}Co source (Gammacell 200/Canada Limited) with a dose rate of 201 rad/min was used to administer the required dose levels. The age of the naupliar stages was the same as the previously irradiated nauplii, 14 ± 6 hr.

To identify the particular stage that was irradiated, individuals from the same cohort as those irradiated were preserved in 5% formalin, 70% alcohol, and 5% glycerin mixture with several drops of buffered formalin added (Gehrs, 1973). The eggs and copepodids were examined microscopically in an attempt to identify the stage of development of the eggs and to determine when the copepodids had reached the adult stage. Adult copepodids can be identified by examining the fifth pair of swimming legs (Czaika, 1968).

RESULTS

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 2.

Egg clutches were dropped by females prior to hatching at dose levels of 0.5 krad and above while no clutches were dropped at the 0.1-krad 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 krad). All clutches in the controls and the 0.1-krad group had some hatching success while all other dose levels had some clutches which experienced 100% mortality.

A t-test was used to test for differences between groups. Significant differences ($P \leq 0.05$) in hatching success between controls and irradiated groups were seen when all clutches were used at dose levels $\geq$ 0.5 krad. Similar results were observed for clutches which were carried until hatching occurred. The percent hatch in dropped clutches decreased with increasing dose. In comparing the percent hatch of clutches in which some hatching occurred, a significant decrease ($P \leq 0.05$) in hatching success with increasing dose was observed at dose levels of 1, 5, and 10 krad; however, at doses of 0.1 and 0.5 krad, the hatching success was not significantly lower than that of the controls. When hatching success was compared between carried clutches and dropped clutches a significant difference ($P < 0.05$) exists in the 1-, 5-, and 10-krad groups with the dropped clutches having a lower percent hatch.

The parameter selected for determining the effects of ionizing radiation on the nauplii of D. clavipes was the development of nauplii to other stages. These developmental stages are listed in Table 3. Nauplii were observed to have reached the Copepodid I stage in all groups which received doses of 0.5 krad and less while all groups receiving 1.0 krad or above suffered 100% mortality. A comparison of the percent of nauplii reaching the copepodid stage at dose levels > 0.5 krad showed an increase in the percentage of developing nauplii with decreasing dose. Similar results were observed for 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), showed 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 krad). In order to test for significant differences in the amount of time required for the 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. Results of the t-test indicated no significant difference in the time it took nauplii

Table 2. *Observed effects of acute ionizing radiation on embryonic* Diaptomus clavipes

Dose (krad)	Percentage of clutches		Percentage hatch ± 95% C. I.			
	Dropped	With no hatch	All clutches	Carried clutches	Dropped clutches	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[a]	63.02 ± 11.6[a]	58.3 ± 0	67.19 ± 10.9
1.0	53.3	20.0	42.57 ± 14.8[a]	65.98 ± 3.2[a]	22.1 ± 17.8	58.04 ± 14.7[a]
5.0	46.7	40.0	27.51 ± 14.4[a]	48.18 ± 15.3[a]	3.9 ± 7.6	55.75 ± 13.9[a]
10.0	46.7	46.7	20.91 ± 10.9[a]	29.11 ± 10.5[a]	6.3 ± 12.4	39.53 ± 10.9[a]

[a] ≤ 0.05 when compared to controls using t-tests for equal sample sizes.

Table 3. Observed effects of acute ionizing radiation on naupliar *Diaptomus clavipes*

Dose (krad)	Percentage			Mean time (days)		
	Nauplii reaching C1[c]	C1 reaching C6[d]	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[a]	39.1[a]	30.0[a]	5.7	12.8	
0.25	66.7[a]	35.0[a]	23.3[a]	5.5	13.8	
0.5	21.6[a]	30.7[a]	13.3[a]	7.4	13.6	7.3 ± 1.9[b]
1.0	0	0	0	0	0	6.6 ± 1.3[b]
5.0	0	0	0	0	0	5.3 ± 0.9[b]
10.0	0	0	0	0	0	3.6 ± 0.7[b]
50.0	0	0	0	0	0	1.8 ± 0.04[b]

[a]0.05 when compared to controls using tests for equality of two percentages.

[b]0.05 when compared to controls using t-tests for equal sample sizes.

[c]First copepodid stage

[d]Sixth copepodid stage, definitive adult

to develop into adults (C-VI). However, the survivorship of nauplii after irradiation was significantly decreased in the 0.5-, 1-, 5-, 10-, and 50-krad groups. The percent survival of the 0.1- and 0.25-krad groups is omitted in Table 3 because of a malfunction of the temperature control in the environmental chamber which caused 100% mortality. The mean survivorship in all groups ranged from 26.4 days for the controls to 1.8 days for the 50-krad group (Fig. 5). After five days, only 7% mortality had occurred in the controls, while after 4 days 100% mortality was observed in the 50-krad group. At day 10, 24% mortality was observed for the controls, while 100% mortality occurred in the 5- and 10-krad groups. The time required for the controls to be reduced 50% was 20 days, while during the same time period, the 0.5- and 1.0-krad groups had suffered 100% mortality.

When organisms in the copepodid stage were tested, the control group as well as the 1-, 5-, and 10-krad groups has some copepodids which developed into adults. In the 50- and 100-krad groups none survived to adulthood (Table 4). Tests for equality of two percentages showed significant differences ($P \leq 0.05$) in the percent of copepodids reaching the adult stage in the 5- and 10-krad groups and results of t-tests showed a significant delay in the time required for development of copepodids into adults. The mean survivorship in all groups ranged from 30.9 to 1.7 days for the control and 100-krad groups, respectively. The survival of copepodids after irradiation was significantly reduced in the 5-, 10-, 50-, and 100-krad groups. No significant difference was observed between controls and 1-krad group.

Survivorship curves for all groups of organisms irradiated as copepodids are shown in Fig. 6. After five days, the control group as well as the 1-, 5-, and 10-krad groups had more than 60% surviving while the 50- and 100-krad groups had suffered 100% mortality by days 3 and 4, respectively. By day 40, 100% mortality occurred in the 5- and 10-krad groups, while more than 20% survived in the control and 1-krad group.

Comparison of the survivorship curves for the adults (Fig. 7) following irradiation revealed that by the 10th day, treatment groups at all dose levels had experienced 14 to 20% mortality while no control animals had perished. No mortality occurred in control animals until day 19 by which time approximately 67% of the 5-krad and 74% of the 10-krad groups had died. Mean survivorship of individuals in the six groups (Table 5) ranged from 47.5 (control) to 18.8 days (10-krad). All treatment groups $\geq$ 1.0 krad showed significantly lower mean survivorship than the controls ($P \leq 0.05$ for 1-krad and $P \leq 0.01$ for 5- and 10-krad groups).

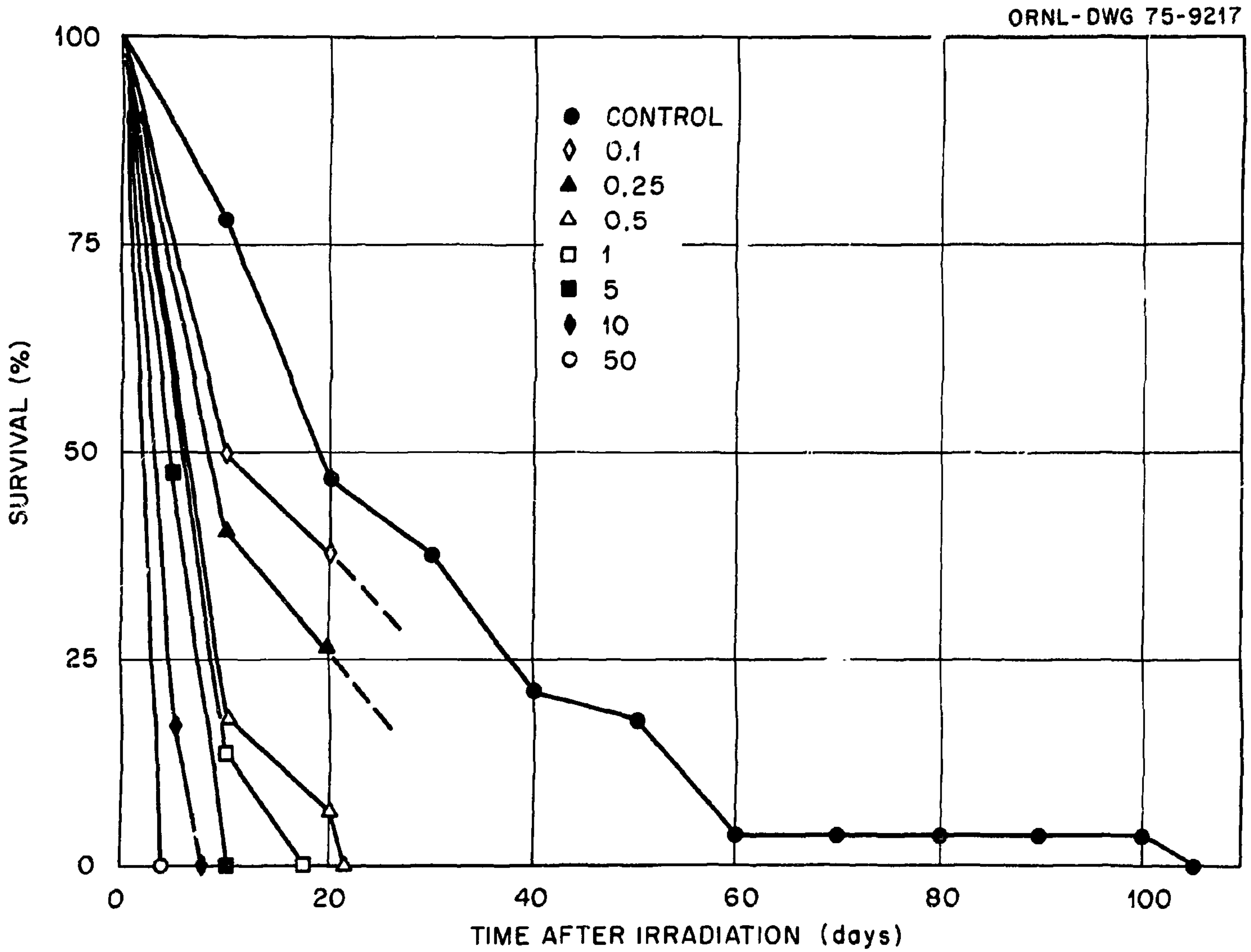

Fig. 5. Survivorship curves for _Diaptomus clavipes_ nauplii following different doses of acute irradiation.

Table 4. Observed effects of acute ionizing radiation on copepodid *Diaptomus clavipes*

Dose (krad)	No. C1 reaching C6	% C1 reaching C6	Mean time (days) to C6 (± 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[a]	19.8 ± 7.6
5.0	4	13.3[b]	16.50 ± 2.9[a]	12.0 ± 4.1[c]
10.0	1	3.3[b]	20.00 ± 0.0[a]	6.5 ± 2.6[c]
50.0	0	0	0	2.3 ± 0.4[c]
100.0	0	0	0	1.7 ± 0.4[c]

P [a]≤ 0.05 when compared to controls using t-tests for unequal sample sizes.

P [b]≤ 0.05 when compared to controls using t-tests for equality of two percentages.

P [c]≤ 0.01 when compared to controls using t-tests for equal sample sizes.

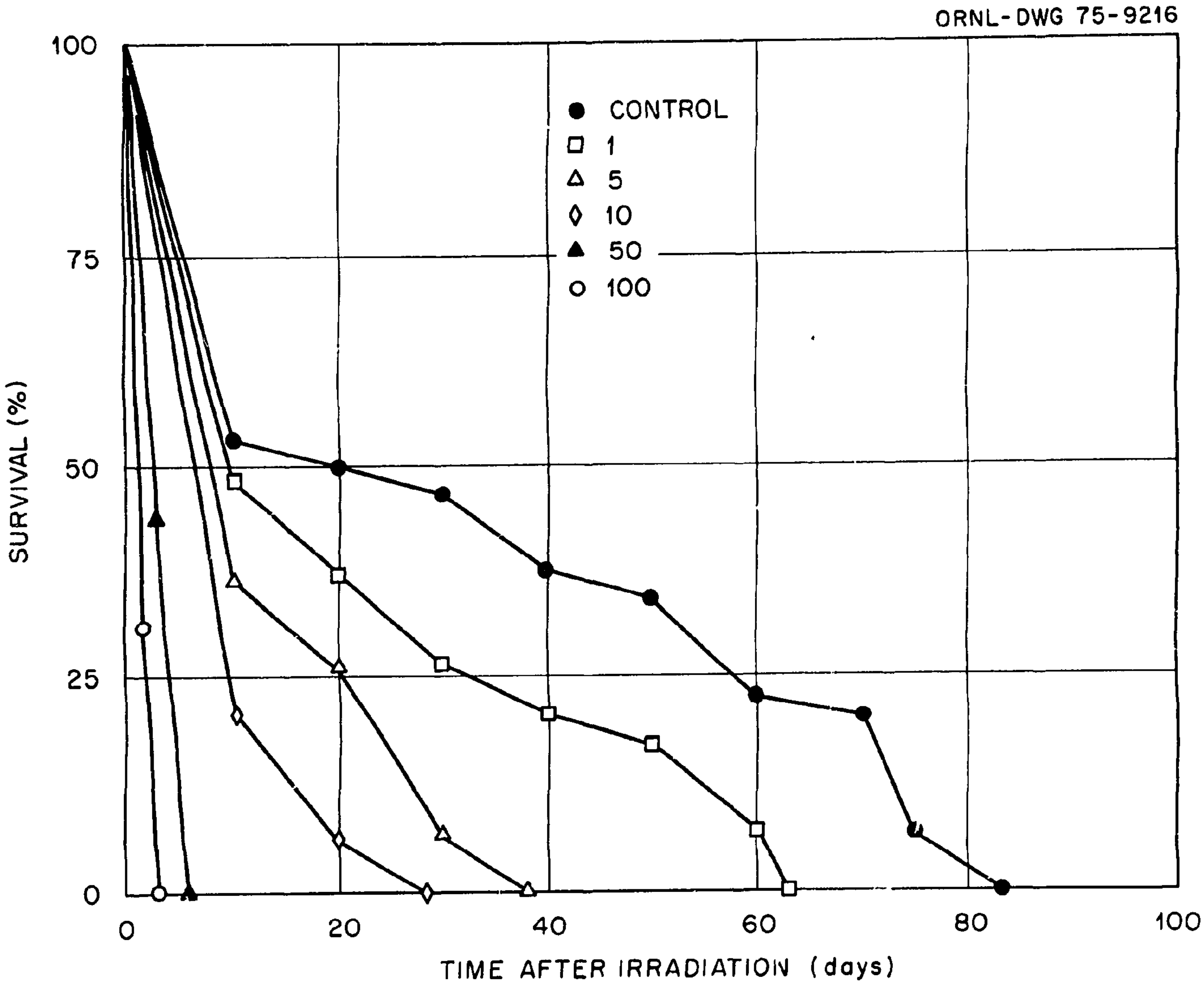

Fig. 6. Survivorship curves for _Diaptomus clavipes_ copepodids following different doses of acute irradiation.

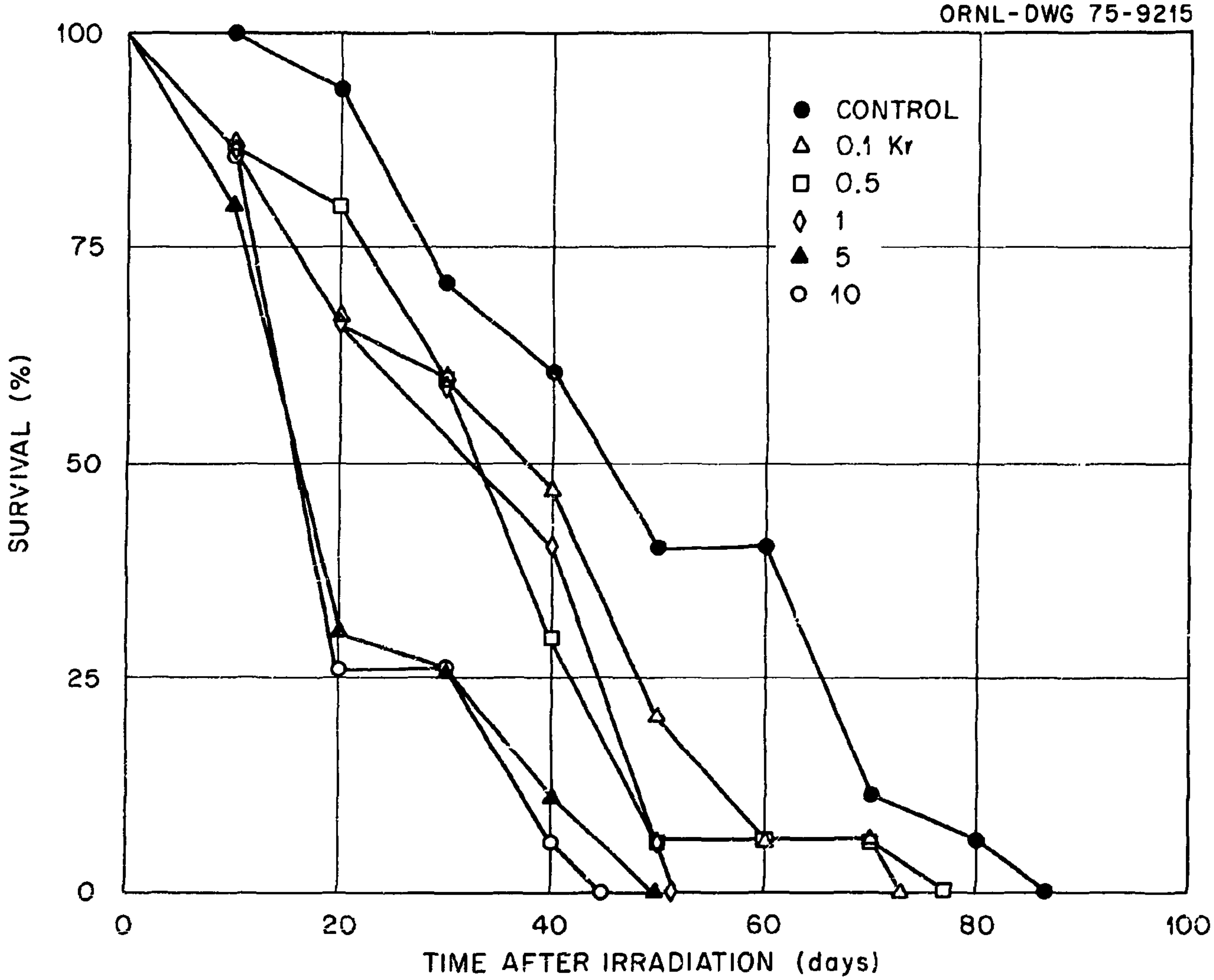

Fig. 7. Survivorship curves for adult female *Diaptomus clavipes* following different doses of acute irradiation.

Table 5. Mean survivorship of adults following different doses of acute ionizing radiation

Dose (krad)	Mean (days) 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

DISCUSSION

The most significant finding of this study was that the naupliar stage had a greater sensitivity to irradiation than the embryonic stage. Significant effects were observed at the 0.1-krad dose level for the naupliar stage and at the 0.5-krad dose level for the embryonic stage. Acute doses of 1- and 5-krad were required to produce a significant effect on the more resistant adult and copepodid stages. In many marine invertebrates and vertebrates, radiation resistance increases with age and is particularly pronounced during embryogenesis and early post-embryogenic life stages (Kinne, 1970). Reichle (1969) found that the past-embryonic stage of the bagworm, Thyridopteryx ephemeraeformis, was more sensitive to irradiation than the late embryonic stage. Several possibilities might explain the unexpected greater sensitivity of the naupliar stage as compared to the embryonic stage.

The first possibility is that the nauplii are more sensitive owing to a greater metabolic activity during the first 24 hr when they are going through several successive molts. It has been established that active cells are more sensitive to ionizing radiation than resting cells or tissues (Kinne, 1970). For example, dry and incapsulated eggs of the brine shrimp Artemia salina have a very low metabolic rate and are more radioresistant than those resuming development after water uptake (Rugh and Clugston, 1955).

Another possibility is that the embryonic stages were irradiated during a later stage which was less radiosensitive. Nakanishi et al., (1964) reported a marked increase of radioresistance with increasing age in embryos of Artemia salina following gamma irradiation from a ^{60}Co source administered at different time intervals up to 15 hr after initiation of development. Kulikov (1966) demonstrated that the radiosensitivity of mollusk embryos decrease substantially from early stages to later stages. The LD_{50} was 450 R for the early blastula stage and 2000 R for the beginning of shell formation. Further evidence of increasing tolerance to ionizing radiation with advancing development of the embryos of the silver salmon Oncorhychus kisutch was demonstrated by 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.

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

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 showed the mean percent hatch of the control, 1- and 10-krad groups to be 72.3, 41.3, and 16.5% respectively. Date in Table 2 show that the mean percent hatchs for the same dose groups are 79.4, 42.6, and 20.9%. The adult survivorship for the control, 1- and 10-krad groups was 49.2, 35.2, and 25.3 days (Gehrs *et al.*, 1975), while the results presented in Table 5 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 remained so for the duration of the irradiation. A marine 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/sec. The dose rate in the present study was well above this level and the dropping of clutches at dose levels $\geq$ 0.5-krad may have resulted from a similar reaction. Another finding in the present study was the substantial difference in hatching success between the carried and dropped clutches at all dose levels $\geq$ 1.0-krad. In the preliminary (Gehrs *et al.*, 1975), it was suggested 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 support this hypothesis.

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 support 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 which indicate 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 data for comparisions with previous studies involving chronic irradiation effects on cladocerans.

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APPENDIX A

Analysis of spring water[a]

Determination	Concentration measured	Method
Potassium	660 ppb[b]	flame emission
Sodium	430 ppb	atomic absorption
Calcium	26.6 ppm[b]	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[b]	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.

[b]ppt = parts per thousand, ppb = parts per billion, ppm = parts per million.

APPENDIX B

Guaranteed analysis of Purina Trout Chow
(size 4) Ralston Purina Company

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, dicaccium 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, chloring chloride, folic acid, pyridoxine lyorochloride, thiamin, niacin, calcium pantothenate, riboflavin supplement, copper sulfate, manganous oxide, zinc oxide, calcium carbonate, cobalt carbonate.