ABSTRACT OF THESIS

THE EFFECTS OF RADIATION AND TEMPERATURE STRESS ON AN AQUATIC ECOSYSTEM

An aquatic microecosystem comprised of Cyclops viridis and Cypris virens was constructed to test the effects of irradiation and temperature stress on these crustaceans, which occupy a vital niche in aquatic food chains. Tests were run on self-regulating populations of an ostracod and cyclops cultured together and cultured separately at three temperature regimes. Adult populations for both were evaluated. It was found that adult ostracods cultured in the absence of cyclops had a longer life span and a greater population density at all three temperature levels than those in mixed cultures. Conversely, cyclops showed greater adult population density when cultured with ostracods. Further tests were run on the effects of temperature stresses of 10 C, 20 C, and 35 C on cyclops and ostracods cultured together. Total net production (all immature forms) and adult population density for both experimental organisms were greater at 10 C than at 20 and 35 C. Furthermore, life span duration for both species was longer at 10 C than at either 20 or 35 C. Net production and adult population density were greater at 20 C than at 35 C. The major portion of the research dealt with the effects of radiation and temperature stress on this aquatic ecosystem. The organisms were irradiated with gamma ray doses of 24, 48, and 96 kr. Two replicas of each exposure level and controls (no irradiation) were cultured at 10 C, 20 C, and 35 C. Adult survival of cyclops was unaffected immediately after radiation exposure at both the 24 and 48 kr level. Cyclops reproduction was inhibited at 16, 24, 48, and 96 kr, but reproduction did occur at 8 kr. The level of irradiation critical to cyclops reproduction is between 8 and 16 kr. The adult population density and life span of cyclops was similar at all radiation levels not exposed to temperature stress. The ostracod survival immediately following irradiation was decreased 15% after exposure to 24 kr; 23% after exposure to 48 kr; and 47% after exposure to 96 kr. Adult population density and net production for both organisms varied insignificantly between 24, 48, and 96 kr at 20 C. However, net production, life span, and adult population density of the irradiated organisms cultured at 35 C were considerably lower than those at 20 C or 10 C. Net production of irradiated organisms was significantly lower than controls; however, net production of irradiated organisms varied very little between radiation levels of 24, 48, and 96 kr.

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5/14/68
Date

THE EFFECTS OF RADIATION AND TEMPERATURE STRESS ON AN AQUATIC ECOSYSTEM

A Thesis
Presented to
the Faculty of the School of Sciences and Mathematics
Morehead State University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science in Biology

by
Gene L. Samsel, Jr.
May 1968

ACKNOWLEDGEMENTS

I wish to acknowledge Dr. M. B. Heaslip, under whose supervision and guidance this experiment was performed. For many valuable and helpful suggestions, I am indebted to Dr. M. E. Pryor and Dr. A. L. Dobson. I am also grateful to Mr. Bruce Harris and Nuclear Engineering Corporation for the use of their facilities. For the assistantship that made possible this study, I am most appreciative to Morehead State University.

Accepted by the faculty of the School of Science and Mathematics, Morehead State University, in partial fulfillment of the requirements for the Master of Science degree.

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THE EFFECTS OF RADIATION AND TEMPERATURE STRESS ON AN AQUATIC ECOSYSTEM

INTRODUCTION

Aquatic microecosystems permit investigations into the effects of radiation and stress factors which would be impossible under field conditions, for the high doses of radiation and amounts of stress required to gain a response from the small aquatic organisms are impractical to use in natural bodies of water(28). Thus, a laboratory model of a simplified ecosystem including two commonly found temperate fresh water invertebrates, an ostracod, *Cypris virens*, and cyclops, *Cyclops viridis*, was constructed. These invertebrates, fundamental links in the food chain of naturally occurring aquatic ecosystems, were chosen as representative organisms to use to evaluate the effects of radiation and temperature stresses on a simplified equilibrated ecosystem. Since these organisms have a vital niche in the basic aquatic food chain, they play a major role in the regulation of the macroorganism production of many aquatic systems. Consequently, the study of environmental factors affecting these organisms deserves investigation.

Radiation modification of aquatic ecosystems and single aquatic organisms have been studied(45, 15, 16, 38, 27, 36, 18, 42, 28, 8). There is some data available on the effect of temperature stress on both single organisms and complex ecosystems(29, 10, 5, 4, 33, 30, 23). However, the joint effects of radiation and temperature on a simplified aquatic ecosystem have not been considered. Experiments were run to determine the combined effects of radiation and temperature stresses on population survival and reproductive potential in a model aquatic ecosystem.

Although the radiation levels to which natural populations will be exposed in the future cannot be predicted, experimental studies of this type will help to establish principles in radiation ecology which will improve our ability to predict the ecological consequences of levels of radiation that might exist(27).

LITERATURE REVIEW

The effects of ionizing radiation on living systems was noted by Muller in 1926 and independently by Stalter in 1931(19). However, biological effects of radiation were not studied widely until radioactive sources became available after World War II. The majority of the early radiation research was concerned with determining lethal doses and deterimental effects for particular species. Some of the first radioecological studies were initiated by Robert Platt and co-workers at Emory University and by E. Odum and co-workers at the University of Georgia. In the mid fifties radioecology gained financial support from government agencies, but it was not until the late fifties and early sixties that natural ecosystems were examined extensively to determine the modifying effects of radiation(18, 36). The effects of temperature stress upon organisms began early in the 1900's and by the advent of radiation ecology in the 1950's, the field had been well explored(29, 10, 5, 41, 33, 30, 23). Little data is available on the effects of temperature stress on ecosystems, and even less is available on aquatic microecosystems(5, 30).

The present investigation includes the effects of radiation and temperature stress on a simplified aquatic microecosystem comprised of *Cyclops viridis* and *Cypris virens*. The ostracod has received little attention beyond study from a taxonomic viewpoint(2, 38). Recent studies of cyclops includes experimentation on reproduction, life cycles, and various factors which have been found to affect adult population survival(11, 14, 32). Edmondson demonstrated that optimum reproductive capacity could be expected from a mature female cyclops when maximum food levels existed(14). In an investigation of the effects of population density on cyclops, Monakov found the optimum number of organisms that could exist in a given volume of water decreased with an increase in density; and overcrowding was found to lower the reproductive rate and increase adult mortality(32). Reading and Sladen found a nonsignificant decrease in the number of cyclops and similar aquatic invertebrates that could exist in distilled water as compared to the number in pond water and noticed that the life span of the organisms in the laboratory environment with distilled water was shorter than their pond water counterparts(40).

Population dynamics, reproductive rates, and population counts were determined by Chernykh for several ecosystems containing daphnia, a fresh water crustacean related to the experimental species(10, 26). Slobodokin studied the energetics of a daphnia population by caloric and biomass equivalents(39). The activity and longevity of daphnia, and related fresh water invertebrates, were shown to increase with optimum nutrition(22). Several biomass determinations of net production have been reported on the relative growth rate of daphnia(39, 27, 10). Beyers(3) determined the net production and assimilation of twelve aquatic ecosystems, and in 1965(4), he determined the rates of photosynthetic and respiratory activity in the same microecosystems. Factors affecting various aquatic ecosystems and the method of calculating the total numbers of the functioning organisms were evaluated(12, 21, 41, 46). Krishnamoorthi showed that although oxygen content had little effect on daphnia and cyclops ecosystems, the same organisms living separately were affected by oxygen content(26). The biomass determination of total productivity is a method used extensively in microecosystem studies(3, 27, 39). Counting of planktonic invertebrates by the direct count method, in which the sample is well shaken and a calculation of total number of adult organisms is determined, showed variation of less than 15% from the biomass population calculation(43); and a direct count method for zooplankton showed a variation of less than 5% when a sample volume included more than 1215 individuals.

In addition to strict developmental aspects, population density, and production counts; considerable research has been performed on the effects of various temperature stresses on aquatic invertebrates and aquatic ecosystems related to the species used in the experimental model(10, 5, 4, 23, 30, 33, 29). McLaren concluded that a decrease in temperature increased the egg size, body size, and fertility of cyclops while decreasing the developmental rate. At higher temperatures the total number of male offspring increased by more than 25%(29). Monakov conducted similar experiments showing that increased temperatures accelerated the incubation period, hatching time, naupliar period, and length of time in each stage from larva to adult, as well as shortening total life span, decreasing brood size, and over-all size of various species of cyclops(32). Reyment's experiments demonstrated that environmental temperature stress thickened the carapace of experimental ostracod(38). Larva and immature forms of daphnia have higher optimum temperatures than adults, and growth rates increase as the temperature varies from 8 to 20 C(23). Daphnia population is larger at 10 and 20 C than at 28 C; and reproduction at higher temperatures is reduced. Tilman and others noted the effects of temperature stress on terrestrial organisms similar in size to those of the experimental model. Temperature variations have fewer effects on flower beetles than on their aquatic counterparts. The effects of temperature on natural and model aquatic ecosystems include Beyers' work of 1962(5) on the relationship between temperature and metabolism and Monakov's studies of the effects of temperature on population size(33). Adult size and life span of zooplankton are negative functions of temperature(30). Spraque noted that resistance of fresh water crustacea to high temperature decreased with size, and that females were more resistant than males(41). Studying the effects of temperature stress on aquatic invertebrates, Beyers found that body metabolism increased with increased temperature(5).

No work has been published on the effects of radiation on cyclops or ostracods. However, data is available on the effects of radiation on the daphnia(28, 27, 42). The chronic irradiation of daphnia at high dose levels yields a reduction in the total number of subsequent generations and individuals in each brood, as well as increasing the time intervals between molting and immature stages reaching sexual maturity(42). Radiation doses below LD 50 showed a stimulating effect on the growth rate of daphnia(42). Marshall investigated the effects of continuous gamma radiation on the intrinsive rate of natural increase of daphnia and found that the increase rate lessened continuously as a nonlinear function of the dose rate(27). Age distribution in proportion to radiation dose showed a decrease in the younger age groups, an increase in older groups, and the intermediate age groups were unaffected. Reduced fertility is the most apparent cause of variations in age groups and life span shortening is secondary. Marshall noted that the exposure time for a given level of irradiation of daphnia had little effect on their net production(28).

Data on radiation effects on less closely related organisms have also been evaluated in an attempt to uncover useful data in this new field of radioecology. Rotifers exposed to 50 kr produced eggs that failed to yield an F 1 generation, and immature egg stages were twenty to fifty per cent more sensitive than their mature counterparts(17). Calkin evaluated the radiation effect data for paramecia(9). Gamma rays have little or no effect on the division and mortality of paramecium a short period after irradiation. "Alpha, beta, gamma, and X rays, showing 100% lethality resulted in the lethal dose ratio of 1:2::5:4:10"(8), respectively of each dose type. Paramecia exposed to doses of 30 to 480 kr showed a reduction in sodium and potassium and an increase in calcium at the higher doses(45). Radiation studies were performed to evaluate the effects of ionizing radiation on planaria, and it was shown that regeneration was suppressed at higher dose levels. Grain mites exposed to a dose of 100 kr showed either no egg hatching or immediate larvae mortality. Larvae are affected at 500 r, but adults are unaffected at 2 kr(6). Two decapod crustacea; females with eggs at time of exposure to the LD 50 dose, failed to release eggs, no hatching occurred after release, or all larvae died within three days if hatching occurred(37).

Several factors affecting radiation modification of single organisms have been evaluated. Daphnia eggs with the lowest water content show the greatest sensitivity to radiation(15). In weevils irradiated with 15 kr, there existed a simple quantitative relation between the concentration of radicals and that of oxygen(7). In a preliminary study in 1963 on the dose-time rate factor of radiation on a biological system, Mole showed that the modification of an aquatic system by a given level of radiation was unaffected by the time of exposure(31).

In 1961, Galbino worked on the biological effects of temperature, pH, moisture, and related stresses following low level irradiation to determine the factors causing reproductive changes in selective organisms related to the experimental model(19). In 1963 and 1964, Kovalena showed that paramecium digestion was suppressed by radiation at temperatures of 4 C and 20 C. These changes in rates of digestion were noted within 15 days at 4 degrees and within 25 days at 20 degrees(25). The general effects of radiation and temperature stress on paramecia show irradiated paramecia adjusted to temperature changes as the character of their RNA was altered(24).

Daphnia and related aquatic invertebrates are the first to bring about fixation of radioactive cesium introduced to the environment(18). Beyers and Odum at present are working on the effects of radiation on a simplified microecosystem at the Atomic Energy Commission Savannah River Plant; however, their results are as yet unpublished. Amer showed an increase in radiation effect on mice with an increase in temperature(1). Ghys (1962) demonstrated that the higher the rate of metabolism of an organism, the greater the effect of radiation(20). Studying the effects on the radiation survival curve, Dewey showed that the greater the diversity of organisms, the greater the survival rate of their populations using an irradiated heterogeneous population of soil micro-organisms(12).

MATERIALS AND METHODS

Pure strains of cyclops, Cyclops viridis, and an ostracod, Cypris virens, were cultured together in wide mouth gallon jugs in distilled water at 20 C to 23 C, with pH ranging from 6.2 to 6.7(34). Oxygen content was not regulated in the system because the oxygen effect on these experimental species is negligible under laboratory conditions(10). The experimental species were fed egg yolk suspended in boiled spring water(44) every three days, allowing ample time for depletion of food, yet keeping optimum food levels at all times. The water level, pH, and temperature of culture jars were checked and regulated periodically. Beyers(4) found that the organisms of a natural crustacean ecosystem adjust to their new environment and become established in a laboratory model within a period of thirty days. The life cycle, egg to adult, is completed in nine days for cyclops and forty-five days for the ostracod.

The laboratory cyclops-ostracod ecosystem became established and attained an equilibrium in thirty days. Five pairs of adult cyclops and three pairs of adult ostracods were then removed, washed in fresh distilled water, and placed in a 100 ml tube containing 75 ml of distilled water and a sprig of elodea. The organisms in each tube were kept at the temperature, pH, and food levels described above. When the organisms in these tubes began to reproduce, three replicas of this simplified ecosystem were subjected to 10 C, 20 C, and 35 C. The three environmental temperatures were maintained under artificial light as follows: 10 C in a refrigerator, 20 C in the laboratory, 35 C in a water bath incubator. The organisms in each tube were fed 1/10 ml of egg suspension every two days and environmental conditions were checked daily. Total counts of the adult population of each tube were recorded every other day. Total population density determined by the direct count method in adults plus immature individuals per milliliter was recorded each week for twelve weeks. Three counts were made for each tube.

The organisms cultured separately were compared with mixed cultures to determine the effects of these three temperature stresses on the population survival and reproductive potential.

The combined effects of radiation and temperature stress on the ecosystem were studied after determining the effects of temperature stress on the model ecosystem. The ostracod and cyclops from equilibrated stock cultures were placed in distilled water in wide mouth pint jars with a sprig of elodea. The organisms were allowed to equilibrate for two weeks and recounted before being irradiated. Two-tenths milliliter of egg suspension was added to each culture for each twenty-four hours of testing, since food could not be added during exposure. Duplicate pint jar cultures were irradiated in a cobalt irradiation chamber located at the Nuclear Engineering Waste Disposal Center at Maxey Flats, Kentucky. Cultures were exposed to 24, 48, and 96 kr of gamma rays at a dose rate of one kr per hour. All six cultures were placed in the irradiated chamber at the same time; two were removed at each exposure time, 24, 48, and 96 hours. Thermoluminescent dosimeters placed on the outside of each jar were removed with the irradiated sample and sent to E. G. and G. P. Corporation, California, for reading. The temperature in the radiation chamber ranged from 10 to 11 C. Duplicate control cultures were kept at the same temperature for 24, 48, and 96 hours in the laboratory. Immediately after irradiation, a direct count was made of the surviving adult population in each irradiated and non-irradiated culture.

Five pairs of cyclops and three pairs of ostracods were removed from each irradiated and control culture and placed in separate 100 ml tubes filled with 75 ml of distilled water and a sprig of elodea. Three replica model ecosystems derived from the irradiated and control cultures were maintained at 10 C, 20 C, and 35 C, as were the cultures in earlier experiments concerned with a single stress factor or temperature. The adult population in each tube was determined each two days for each temperature level and the total production was calculated by direct count of three 1 ml samples per culture after the adults were removed for the temperature stress experimentation described above. Survival of adult and immature stages, life span determination, time interval between generations and productivity of surviving adults were recorded for twelve weeks. Comparisons between groups were recorded and graphically analyzed.

EXPERIMENTAL RESULTS

This study was conducted to determine the effects of gamma radiation with temperature stress on a simplified aquatic ecosystem. The study was divided into two basic parts: (1) testing the effects of temperature stress on aquatic organisms living separately and together (Fig. I, II, III; Table I, II, III), and (2) evaluating the effects of irradiation followed by temperature stress on aquatic crustacea living in a ecosystem (Fig. IV, V, VI, VII, VIII, IX; Table IV, V). Net production (measure of numbers of immature stages of both experimental species) and adult population density were the criteria used to evaluate the effects of temperature stress, and radiation followed by temperature stress. Cyclops viridis has a life cycle of 25 days at 20 C and completes the reproductive cycle (eggs to larva) in five to nine days. The ostracod, Cypris virens, has a life cycle of 70 days and completes the reproductive cycle in 15 to 25 days. In addition to data collected for the two basic parts in this study, the following information was collected weekly and used to aid in the evaluation of the effects of temperature and/or radiation on an aquatic ecosystem.

(1) Only the adults from the irradiated culture were exposed to temperature stress. The immature organisms in these cultures were kept at 20 C following irradiation and their net production was compared with the unirradiated culture of immature stage exposed to the same temperature.

(2) Numbers of adults surviving irradiation were recorded and compared with numbers of adults from nonirradiated cultures (Fig. XI, Table VII).

(3) An additional study was performed to determine the critical radiation dose level for cyclops reproduction.

At all temperature regimes it was noted that the adult population density of the ostracod was greater when the organisms were grown separately as compared with those grown with cyclops. Conversely, the adult population density of cyclops was less when grown alone than when cultured with ostracods (Fig. I; Table I).

Similarly, the adult population density of cyclops and the ostracod grown together was greatest at the cold temperature regime for both experimental species. The ostracod showed a greater life span at lower temperatures. Population densities at medium and high temperature levels for both cyclops and ostracods were similar. Life span was greatest at the high temperature for ostracods and greatest at the medium temperature for cyclops (Fig. III; Table III).

Total net production of irradiated cyclops and ostracods grown together was greater at the cold temperature regime, and least at the high temperature level (Fig. IV; Table IV). Since the cyclops did not reproduce following irradiation, net production studies were made on the ostracod only. The critical level for cyclops reproduction was found to be between 8 and 16 kr. Adult population density of irradiated cyclops and ostracod grown together was similar at medium and cold temperature levels, as was life span duration; however, there was a definite decrease in both density and life span of irradiated experimental species at the high temperature regime (Fig. V; Table V).

Total net production of cyclops and ostracod in nonirradiated cultures showed an initial rise and gradually leveled off to 1200/ml by the end of the seventh week. The net production of organisms in irradiated cultures decreased continually to 400 per ml after exposure to all three radiation levels by the end of the seventh week (Fig. VI; Table VI).

There was no significant adult mortality immediately following exposure of cyclops irradiated at 24 and 48 kr of gamma rays. However, at the 96 kr level there was a significant decrease in adult cyclops mortality when compared with mortality of nonirradiated cyclops. Ostracod mortality, although not greatly affected by 24 kr of gamma rays, showed a significant decrease at 48 and 96 kr when compared to nonirradiated ostracod mortality.

TABLE I

Adult Population Density of *Cyclops viridis* and *Cypris virens* Cultured Separately Under Three Temperature Regimes

		TEMPERATURE REGIMES		
No. of Weeks	Organism	35° C	20° C	10° C
1	Cyclops viridis	4	4	4
	Cypris virens	4	4	4
2	Cyclops viridis	4	4	4
	Cypris virens	4	4	4
3	Cyclops viridis	4	4	4
	Cypris virens	4	4	4
4	Cyclops viridis	3	4	4
	Cypris virens	4	4	4
5	Cyclops viridis	1	25	0
	Cypris virens	4	4	4
6	Cyclops viridis	0	25	0
	Cypris virens	4	4	4
7	Cyclops viridis	0	5	5
	Cypris virens	0	4	4
8	Cyclops viridis	0	4	5
	Cypris virens	0	3	4
9	Cyclops viridis	0	10	10
	Cypris virens	0	2	3

FIGURE I

Mean Adult Population Density of _Cyclops viridis_ and _Cypris virens_ Cultured Separately and Together Under Three Temperature Regimes

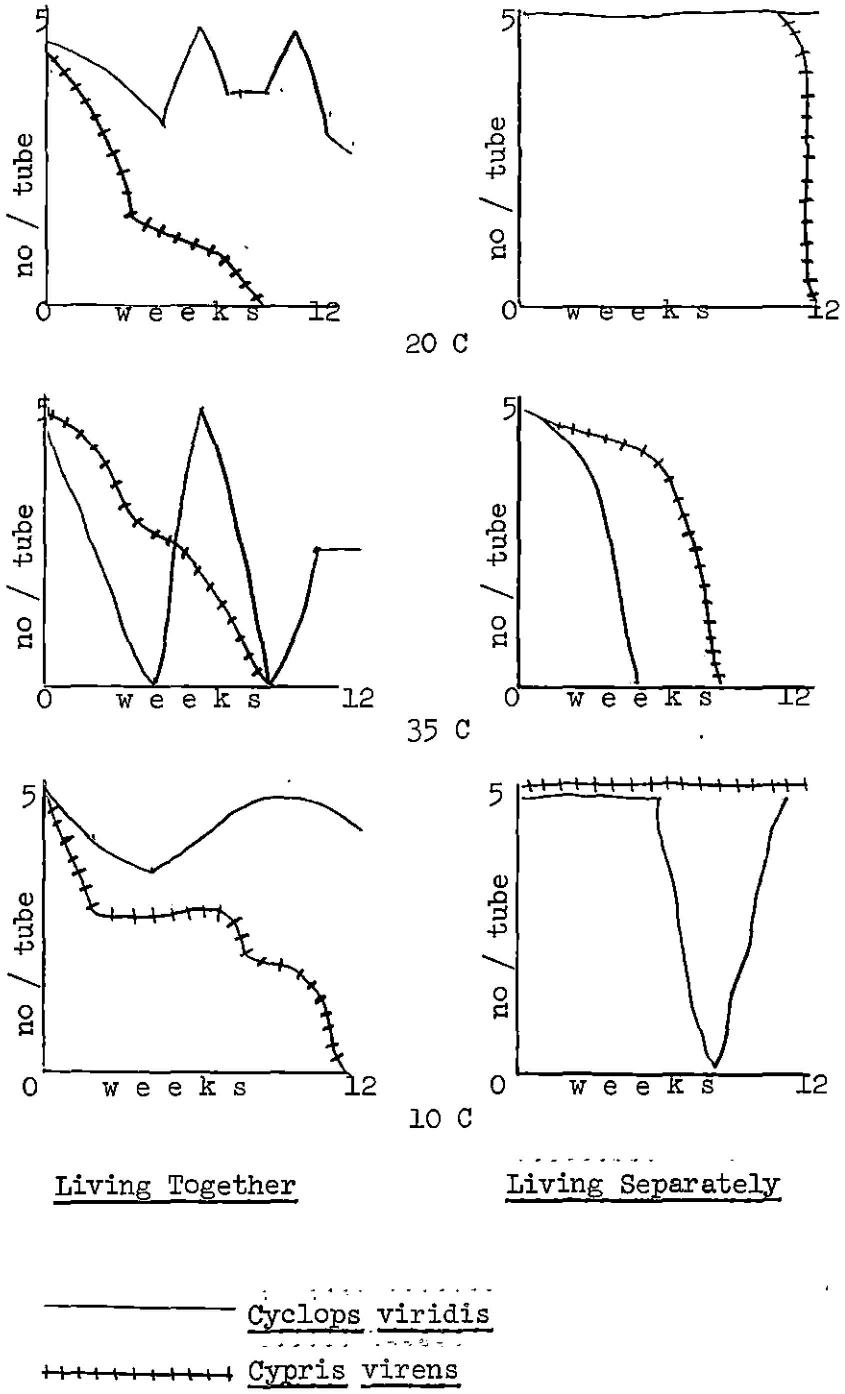


TABLE II

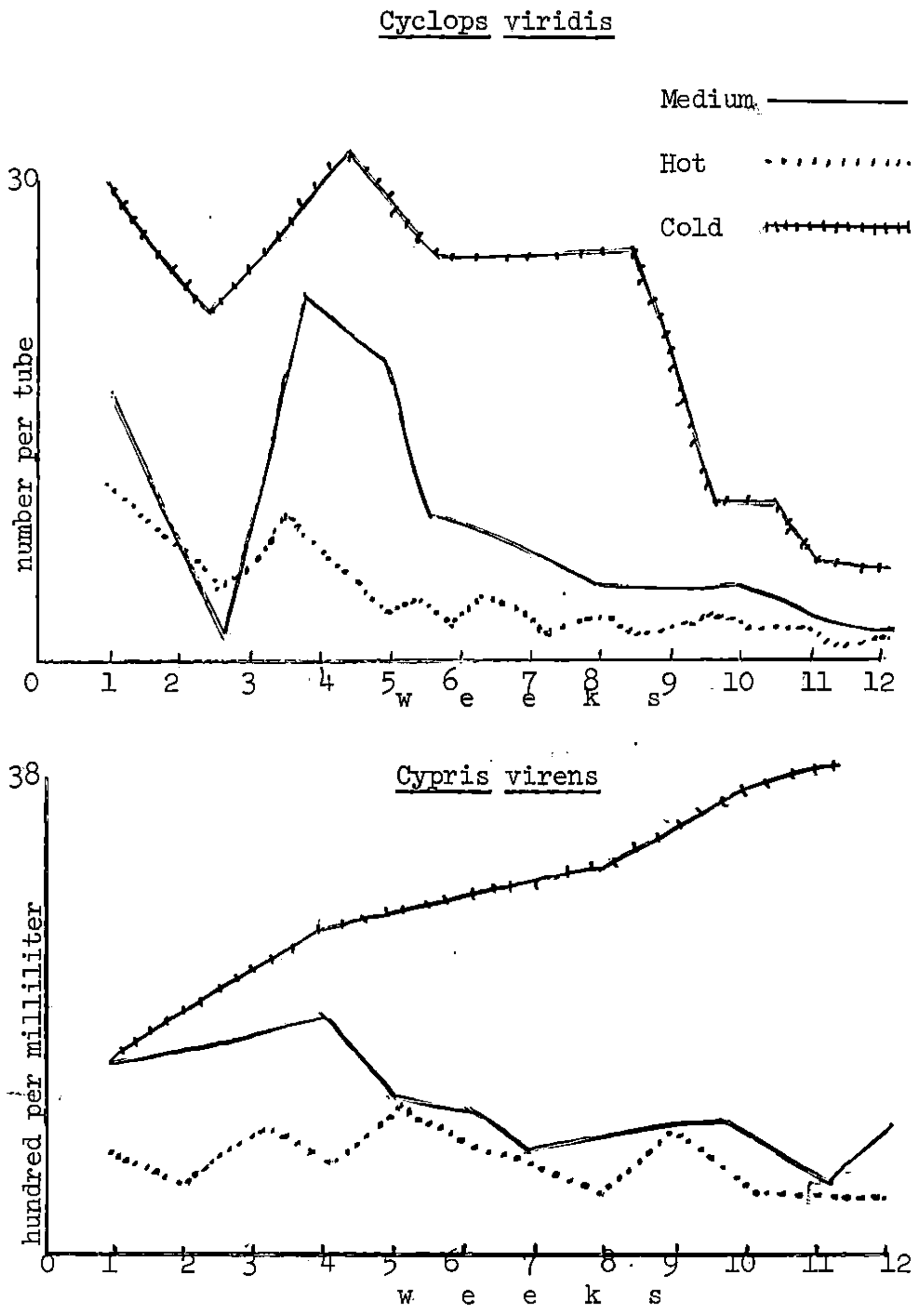
Net Production in Three Replicas of *Cyclops viridis* and *Cypris virens*

Cultured Together at Three Temperature Regimes

		TEMPERATURE REGIMES								
		10 C			20 C			35 C		
No. of Weeks		1	2	3	4	5	6	7	8	9
0	Cyclops viridis	0	0	0	0	0	0	0	0	0
	Cypris virens	0	0	0	0	0	0	0	0	0
1	Cyclops viridis	10	50	50	10	10	20	0	10	20
	Cypris virens	1080/ml	1620/ml	1080/ml	540/ml	1360/ml	1800/ml	720/ml	720/ml	360/ml
2	Cyclops viridis	3	30	30	1	1	1	4	3	8
	Cypris virens	1500/ml	2000/ml	1800/ml	740/ml	1800/ml	1400/ml	720/ml	720/ml	450/ml
3	Cyclops viridis	4	20	60	3	0	60	4	4	13
	Cypris virens	2000/ml	2200/ml	2200/ml	1400/ml	1800/ml	1900/ml	600/ml	800/ml	800/ml
4	Cyclops viridis	35	30	60	6	4	45	0	3	9
	cypris virens	2900/ml	2300/ml	2100/ml	1800/ml	1950/ml	1900/ml	500/ml	500/ml	1100/ml
5	Cyclops viridis	16	18	40	5	4	16	0	0	7
	Cypris virens	2500/ml	2500/ml	2500/ml	1500/ml	1000/ml	1500/ml	1000/ml	1150/ml	1700/ml
6	Cyclops viridis	30	15	35	2	3	20	0	8	1
	Cypris virens	2700/ml	2200/ml	3000/ml	1700/ml	1000/ml	1300/ml	500/ml	1150/ml	500/ml
7	Cyclops viridis	0	25	25	0	1	20	0	5	8
	Cypris virens	5000/ml	2200/ml	2200/ml	1100/ml	700/ml	700/ml	500/ml	1100/ml	1100/ml
8	Cyclops viridis	0	15	15	1	1	20	0	3	4
	Cypris virens	6000/ml	2500/ml	2200/ml	1000/ml	800/ml	1000/ml	400/ml	900/ml	900/ml
9	Cyclops viridis	10	15	15	0	1	20	0	0	0
	Cypris virens	8000/ml	3000/ml	2200/ml	1000/ml	800/ml	1300/ml	200/ml	700/ml	800/ml

FIGURE II

Net Production in Three Replicas of *Cyclops viridis* and *Cypris virens* Cultured Together at Three Temperature Regimes



Cyclops viridis

Cypris virens

TABLE III

Adult Population Density of Three Replicas of *Cyclops viridis* and *Cypris virens* Cultured Together at Three Temperature Regimes

		TEMPERATURE REGIMES								
		10° C			20° C			35° C		
No. of Weeks	Organism	1	2	3	4	5	6	7	8	9
0	Cyclops viridis	5	5	5	5	5	5	5	5	5
	Cypris virens	5	5	5	5	5	5	5	5	5
1	Cyclops viridis	5	5	5	5	5	5	5	5	5
	Cypris virens	5	5	5	5	5	5	5	5	5
2	Cyclops viridis	5	4	4	4	4	4	2	3	4
	Cypris virens	4	3	3	3	2	2	3	4	3
3	Cyclops viridis	5	4	4	4	4	3	4	0	4
	Cypris virens	3	3	2	2	1	1	2	2	1
4	Cyclops viridis	5	4	7	7	3	10	4	6	8
	Cypris virens	3	3	1	1	0	0	2	2	1
5	Cyclops viridis	8	5	6	6	3	8	6	3	3
	Cypris virens	3	3	1	1	0	1	2	2	1
6	Cyclops viridis	6	8	5	5	4	8	0	0	2
	Cypris virens	1	3	0	0	0	0	2	1	0
7	Cyclops viridis	6	8	2	2	3	10	0	2	8
	Cypris virens	0	3	0	0	0	0	2	0	0
8	Cyclops viridis	10	10	1	1	1	10	0	2	8
	Cypris virens	0	2	0	0	0	0	0	0	0
9	Cyclops viridis	10	10	1	1	1	8	0	2	4
	Cypris virens	0	2	0	0	0	0	0	0	0
10	Cyclops viridis	9	10	0	0	1	10	0	0	0
	Cypris virens	0	10	0	0	0	0	0	0	0

FIGURE III

Mean Adult Population Density of Cyclops viridis and Cypris virens Grown Together at Three Temperature Regimes

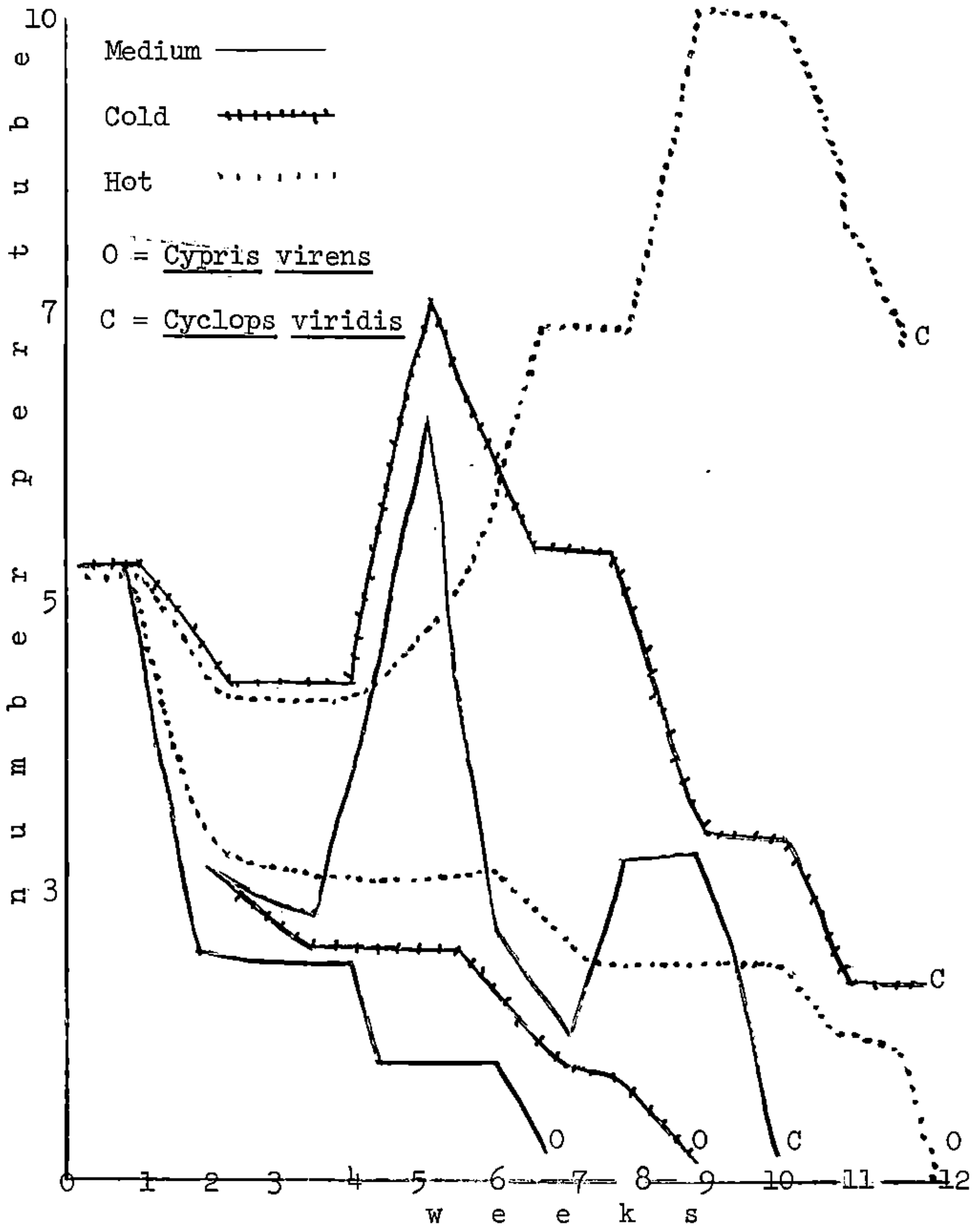


TABLE IV

Total Net Production of Gamma Irradiated *Cyclops viridis* and *Cypris virens* Cultured Together Under Three Temperature Regimes

	TEMPERATURE REGIMES								
	35°			20°			10°		
No. of Weeks	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr
0 Control									
Cyclops viridis	0								
Cypris virens	1500/ml	1500/ml	0	1800/ml	1650/ml	1800/ml	2200/ml	1800/ml	0
Irradiated									
Cyclops viridis	0	0	0	25	20	0	0	0	0
Cypris virens	1650/ml	1650/ml	0	2000/ml	1850/ml	900/ml	2600/ml	1400/ml	0
1 Control									
Cyclops viridis	0	0	0	25	20	0	0	0	0
Cypris virens	1500/ml	1500/ml	0	1800/ml	1650/ml	1800/ml	2200/ml	1800/ml	0
Irradiated									
Cyclops viridis	0	0	0	0	0	0	0	0	0
Cypris virens	1650/ml	1650/ml	0	2000/ml	1850/ml	900/ml	2600/ml	1400/ml	0
2 Control									
Cyclops viridis	0	0	0	60	60	10	0	10	0
Cypris virens	1500/ml	1500/ml	1500/ml	1600/ml	1500/ml	900/ml	2300/ml	1650/ml	1800/ml
Irradiated									
Cyclops viridis	0	0	0	0	0	0	0	0	0
Cypris virens	1375/ml	1350/ml	800/ml	1525/ml	1400/ml	850/ml	2100/ml	650/ml	1000/ml

TABLE IV (continued)

No. of Weeks	35°			20°			10°		
	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr
3 Control									
Cyclops viridis	0	0	0	30	25	60	0	0	0
Cypris virens	850/ml	1100/ml	850/ml	1900/ml	1600/ml	850/ml	2100/ml	1600/ml	1300/ml
Irradiated									
Cyclops viridis	0	0	0	0	0	0	0	0	0
Cypris virens	825/ml	800/ml	575/ml	1100/ml	650/ml	750/ml	2400/ml	1400/ml	1100/ml
4 Control									
Cyclops viridis	0	0	0	20	35	16	0	30	0
Cypris virens	1100/ml	1000/ml	1100/ml	2100/ml	2500/ml	1750/ml	2800/ml	1750/ml	1300/ml
Irradiated									
Cyclops viridis	0	0	0	0	0	0	0	0	0
Cypris virens	1050/ml	500/ml	400/ml	1050/ml	800/ml	1150/ml	2900/ml	1000/ml	800/ml
5 Control									
Cyclops viridis	0	0	0	30	30	15	0	30	0
Cypris virens	1000/ml	1300/ml	1000/ml	2500/ml	1800/ml	1100/ml	2500/ml	1800/ml	2000/ml
Irradiated									
Cyclops viridis	0	0	0	0	0	0	0	0	0
Cypris virens	100/ml	800/ml	700/ml	750/ml	600/ml	550/ml	2200/ml	1800/ml	1700/ml
6 Control									
Cyclops viridis	0	0	0	30	30	15	0	40	0
Cypris virens	800/ml	1100/ml	1300/ml	2200/ml	2000/ml	900/ml	2500/ml	2400/ml	2400/ml
Irradiated									
Cyclops viridis	0	0	0	0	0	*	0	0	0
Cypris virens	600/ml	750/ml	1150/ml	900/ml	1150/ml		2900/ml	2400/ml	2400/ml
7 Control									
Cyclops viridis	0	0	0	0	0	0	0	0	0
Cypris virens	1200/ml	1200/ml	1100/ml	2500/ml	1700/ml	1700/ml	2800/ml	2800/ml	1700/ml
Irradiated									
Cyclops viridis	0	0	0	0	0	0	0	0	0
Cyrpis virens	1050/ml	1050/ml	1100/ml	1200/ml	800/ml	800/ml	3600/ml	4000/ml	3100/ml

* = Contaminated

FIGURE IV

Mean Net Production of Cyclops viridis and Cypris virens Cultured Together Under Three Temperature Regimes Following Exposure to 0 or 24 kr Gamma Rays

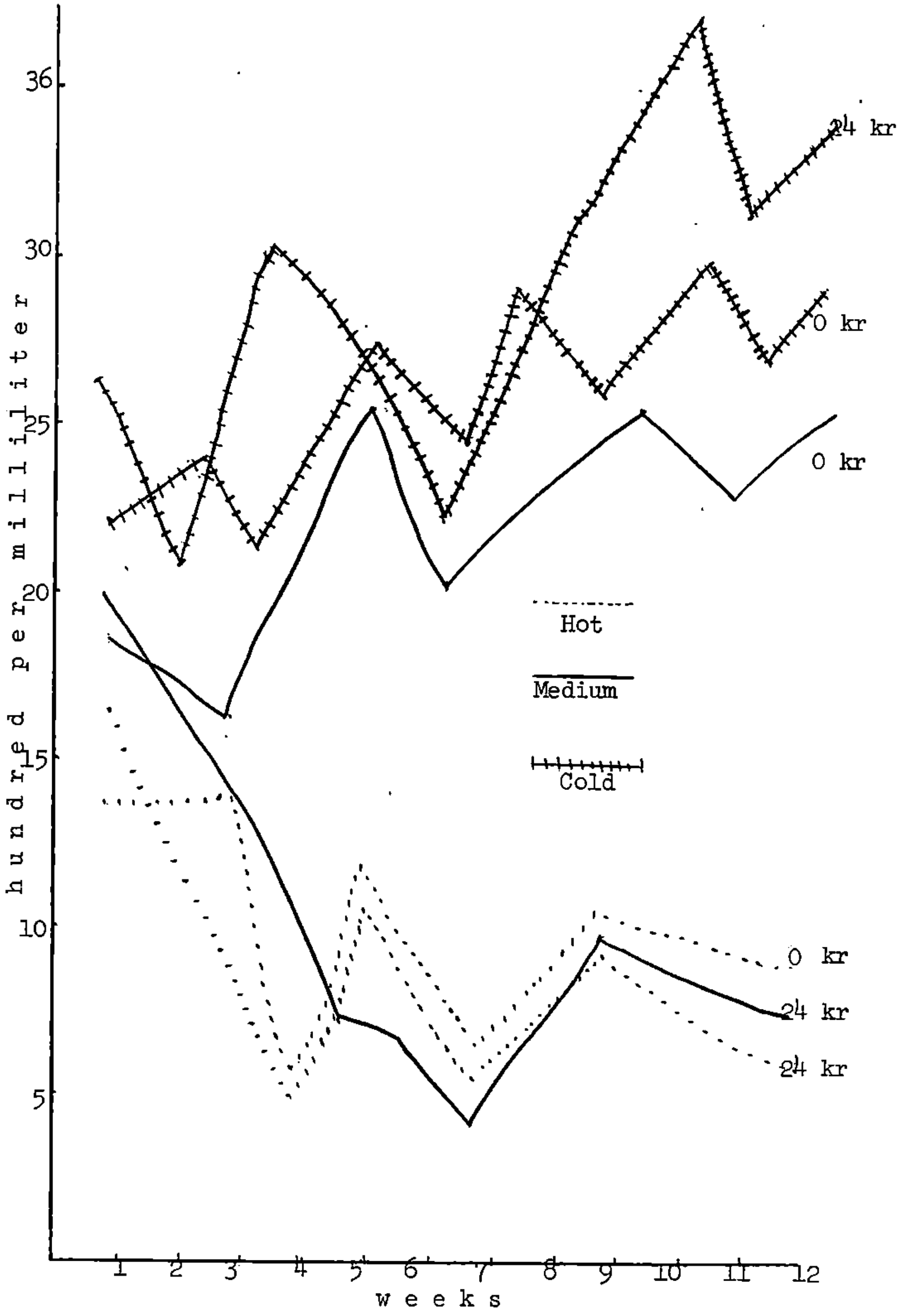


FIGURE V

Mean Net Production of *Cyclops viridis* and *Cypris virens* Cultured Together Under Three Temperature Regimes Following Exposure to 0 or 48 kr Gamma Rays

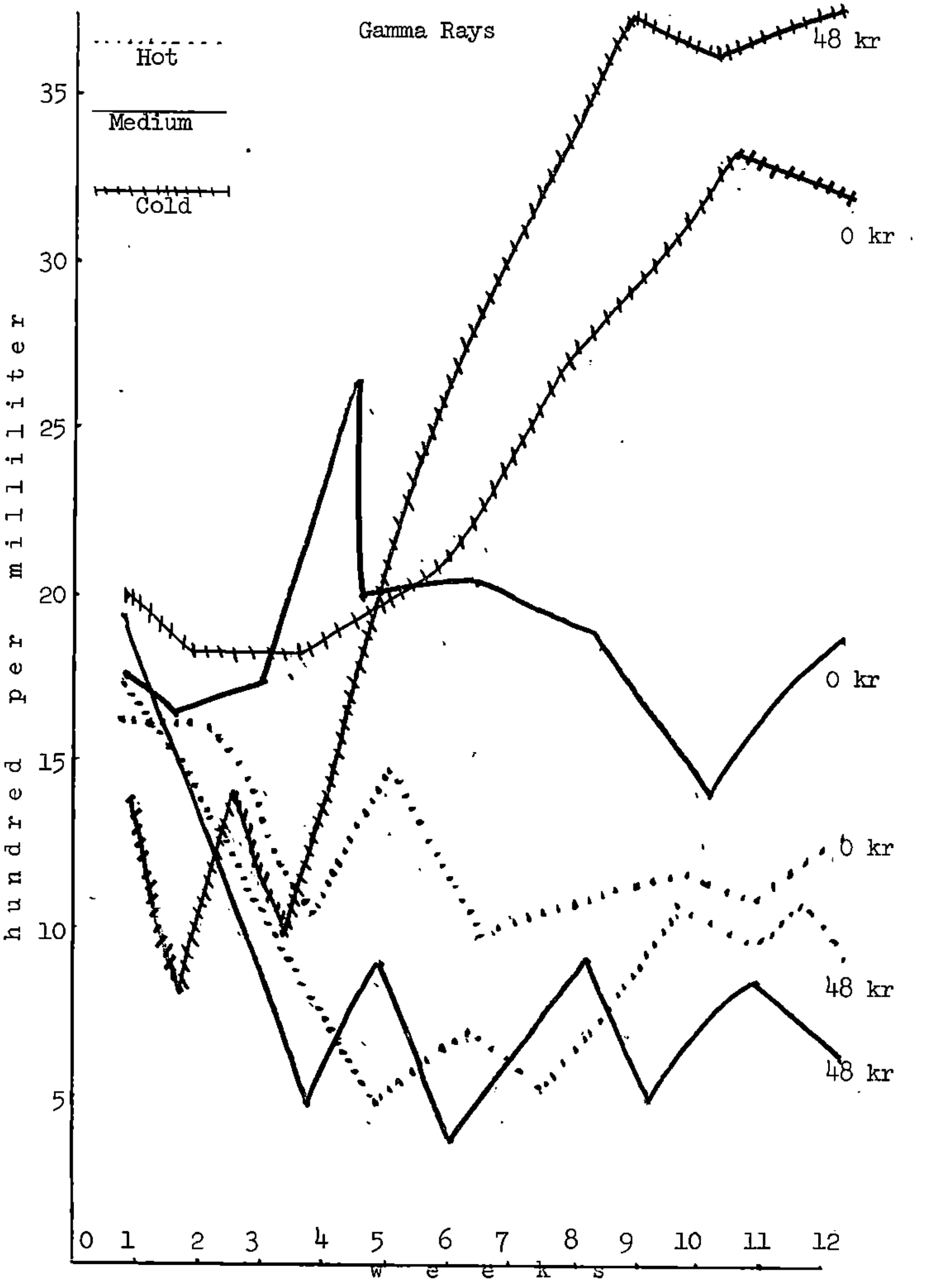


FIGURE.VI

Mean Net Production of *Cyclops viridis* and *Cypris virens* Cultured Together Under Three Temperature Regimes Following Exposure to 0 or 96 kr Gamma Rays

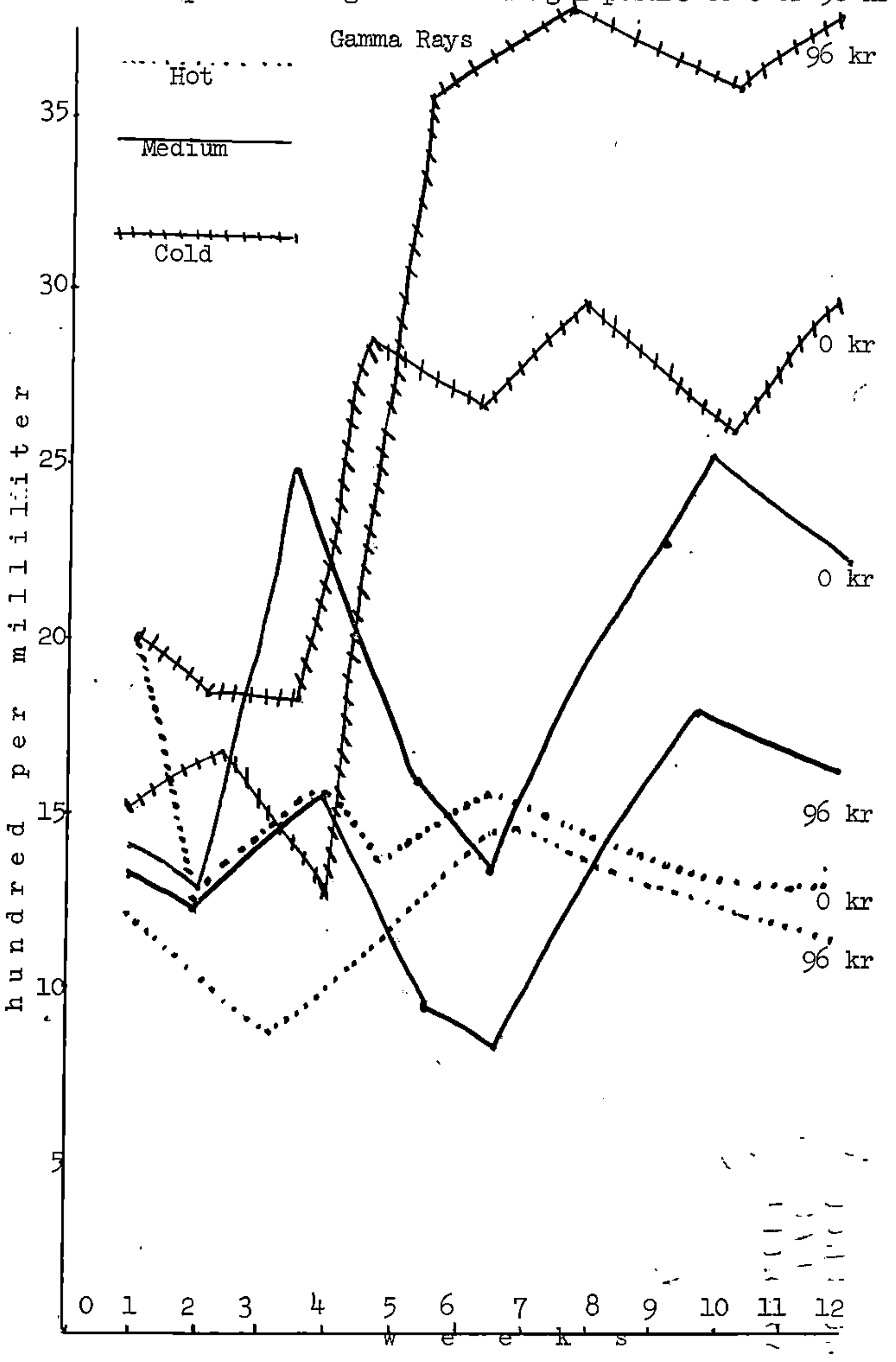


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TABLE Va

Adult Population Density of *Cyclops viridis* and *Cypris virens* Grown Together Under Three Temperature Regimes

CONTROLS

		TEMPERATURE								
		35 C			20 C			10 C		
No. of Weeks	Organism	24	48	96	24	48	96	24	49	96
0	Cyclops viridis	10	10	0	10	10	0	10	10	0
	Cypris virens	5	5	0	5	5	0	5	5	0
1	Cyclops viridis	10	10	0	10	10	0	10	10	0
	Cypris virens	5	5	0	5	5	0	5	5	0
2	Cyclops viridis	10	10	10	10	10	10	10	10	10
	Cypris virens	4	4	5	5	5	5	5	5	5
3	Cyclops viridis	10	10	10	10	10	10	10	10	10
	Cypris virens	1	1	2	4	4	5	4	5	5
4	Cyclops viridis	6	4	3	10	10	10	8	6	8
	Cypris virens	0	1	1	3	3	4	3	4	4
5	Cyclops viridis	0	1	1	10	10	10	4	6	5
	Cypris virens	0	1	0	2	2	4	3	3	4
6	Cyclops viridis	0	0	0	10	10	10	8	10	10
	Cypris virens	0	0	0	2	1	3	2	1	4
7	Cyclops viridis	0	0	0	10	10	8	4	4	2
	Cypris virens	0	0	0	1	1	1	1	1	1
8	Cyclops viridis	0	0	0	10	10	10	6	10	2
	Cyclops virens	0	0	0	0	0	1	0	0	0
9	Cyclops viridis	0	0	0	2	10	0	2	1	0
	Cyclops virens	0	0	0	0	10	0	0	0	0

TABLE V

Adult Population Density of Gamma Irradiated Cyclops viridis and Cypris virens Grown Together Under Three Temperature Regimes

No. of Weeks	TEMPERATURE REGIMES								
	35 C			20 C			10 C		
	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr
0									
Replica 1:									
Cyclops viridis	10	10	0	10	10	0	10	10	0
Cypris virens	5	5	0	5	5	0	5	5	0
Replica 2:									
Cyclops viridis	10	10	0	10	10	0	10	10	0
Cypris virens	4	4	5	5	5	5	5	5	5
1									
Replica 1:									
Cyclops viridis	10	10	0	10	10	0	10	10	0
Cypris virens	5	5	0	5	5	0	5	5	0
Replica 2:									
Cyclops viridis	10	10	0	10	10	0	10	10	0
Cypris virens	5	5	0	5	5	0	5	5	0
2									
Replica 1:									
Cyclops viridis	10	10	10	10	10	10	10	10	10
Cypris virens	3	3	5	4	3	5	5	3	5
Replica 2:									
Cyclops viridis	10	10	10	10	10	10	10	10	10
Cypris virens	3	2	5	4	4	5	5	3	5
3									
Replica 1:									
Cyclops viridis	10	10	0	4	10	8	10	10	9
Cypris virens	3	2	2	3	3	4	3	2	5
Replica 2:									
Cyclops viridis	10	10	8	8	10	10	10	10	9
Cypris virens	2	2	3	3	3	3	3	4	5

TABLE V (continued)

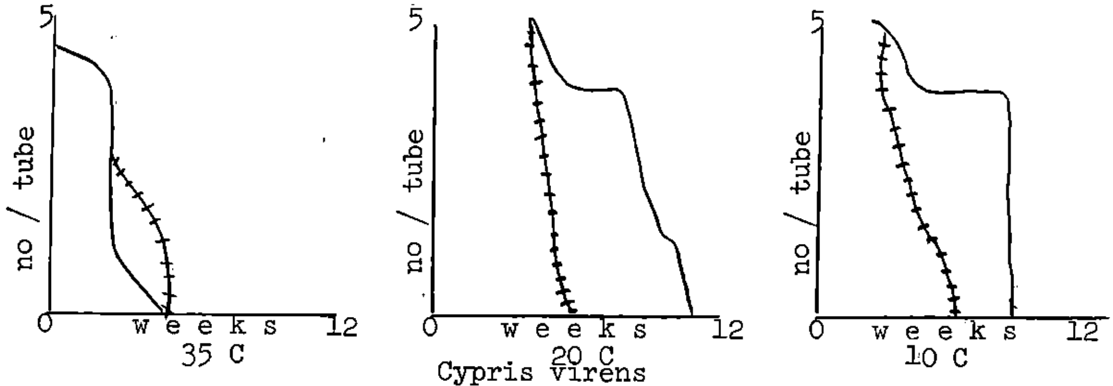
No. of	35 C			20 C			10 C		
Weeks	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr
4									
Replica 1:									
Cyclops viridis	2	3	0	2	2	5	2	5	6
Cypris virens	3	1	2	2	3	2	2	2	1
Replica 2:									
Cyclops viridis	0	1	0	2	2	3	2	5	6
Cypris virens	1	2	2	2	3	3	1	4	3
5									
Replica 1:									
Cyclops viridis	0	0	0	2	1	2	2	3	3
Cypris virens	1	0	0	2	3	0	2	1	1
Replica 2:									
Cyclops viridis	0	0	0	2	1	1	2	2	2
Cypris virens	1	2	0	2	2	2	0	2	2
6									
Replica 1:									
Cyclops viridis	0	0	0	2	2	0	2	2	2
Cypris virens	1	0	0	2	3	2	1	0	0
Replica 2:									
Cyclops viridis	0	0	0	2	2	0	2	2	2
Cyrpis virens	0	0	0	2	2	10	0	1	0
7									
Replica 1:									
Cyclops viridis	0	0	0	2	2	0	2	2	0
Cypris virens	0	0	0	1	1	2	0	0	0
Replica 2:									
Cyclops viridis	0	0	0	2	2	0	2	2	0
Cypris virens	0	0	0	1	1	0	0	1	0
8									
Replica 1:									
Cyclops viridis	0	0	0	10	10	0	2	1	2
Cypris virens	0	0	0	0	0	1	0	0	0
Replica 2:									
Cyclops viridis	0	0	0	10	1	0	2	1	2
Cypris virens	0	0	0	0	0	1	0	0	0

TABLE V (continued)

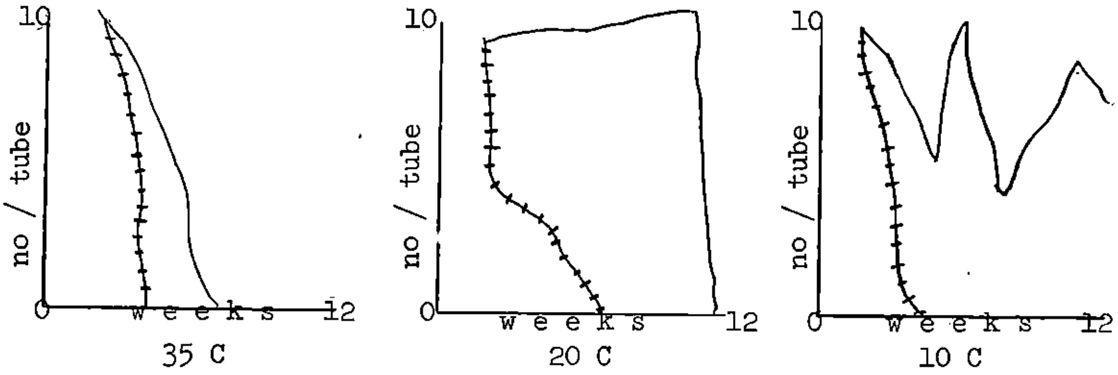
No. of Weeks	35 C			20 C			10 C		
	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr	24 kr	48 kr	96 kr
9									
Replica 1:									
Cyclops viridis	0	0	0	2	0	0	2	1	0
Cypris virens	0	0	0	0	10	0	0	0	0
Replica 2:									
Cyclops viridis	0	0	0	2	0	0	2	1	0
Cypris virens	0	0	0	0	10	0	0	0	0

FIGURE VII

Mean Adult Population Density of <u>Cyclops viridis</u> and <u>Cypris virens</u> Exposed to 0 or 24 kr of Gamma Rays and Cultured Together Under Three Temperature Regimes



<u>Cypris</u> <u>virens</u>



<u>Cyclops</u> <u>viridis</u>

——— 0 kr

┼┼┼┼┼┼┼┼┼┼ 24 kr

FIGURE VIII

Mean Adult Population Density of <u>Cyclops viridis</u> and <u>Cypris virens</u>

Exposed to 0 or 48 kr of Gamma Rays and Cultured Together

Under Three Temperature Regimes

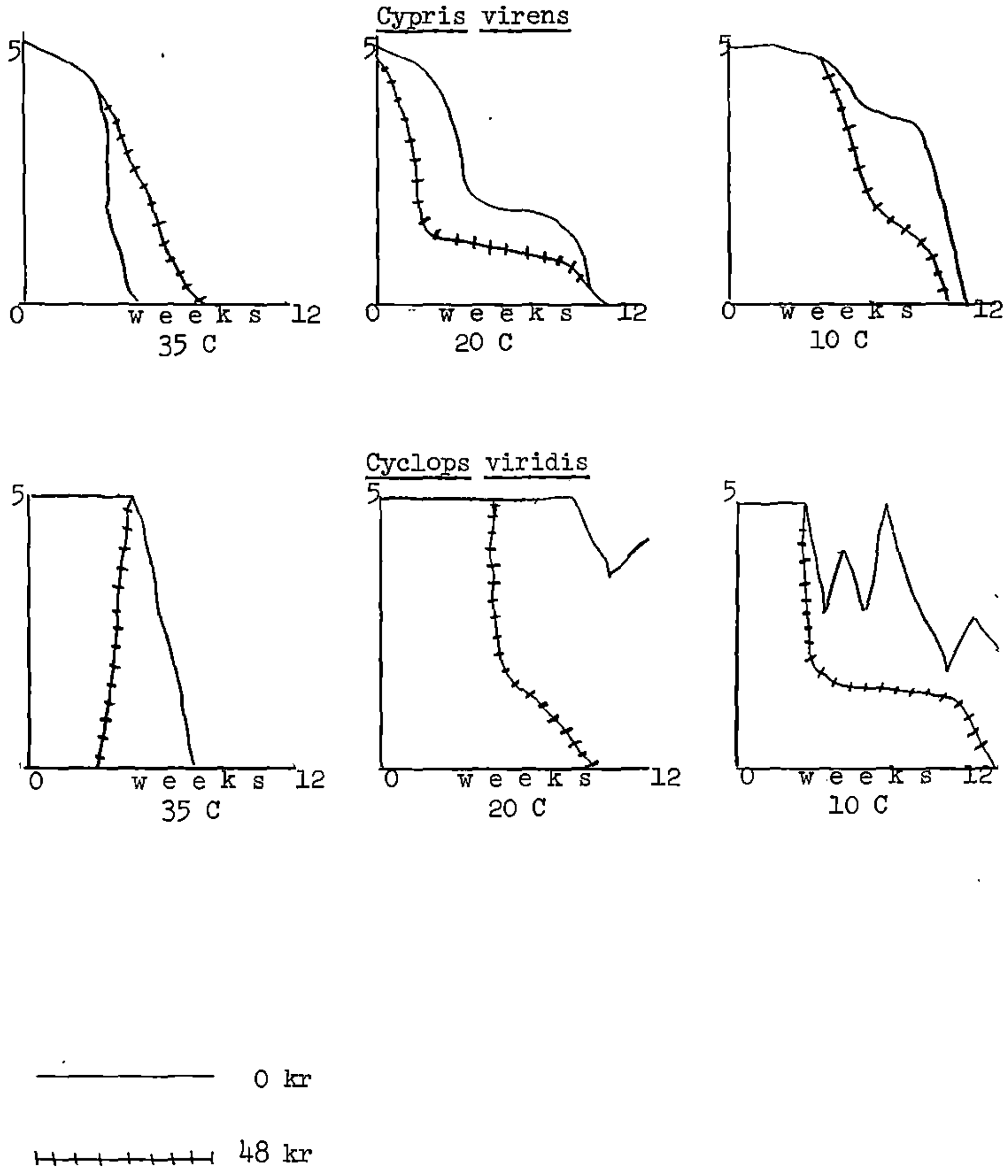


FIGURE IX

Mean Adult Population Density of *Cyclops viridis* and *Cypris virens* Exposed to 0 or 96 kr of Gamma Rays and Cultured Together Under Three Temperature Regimes

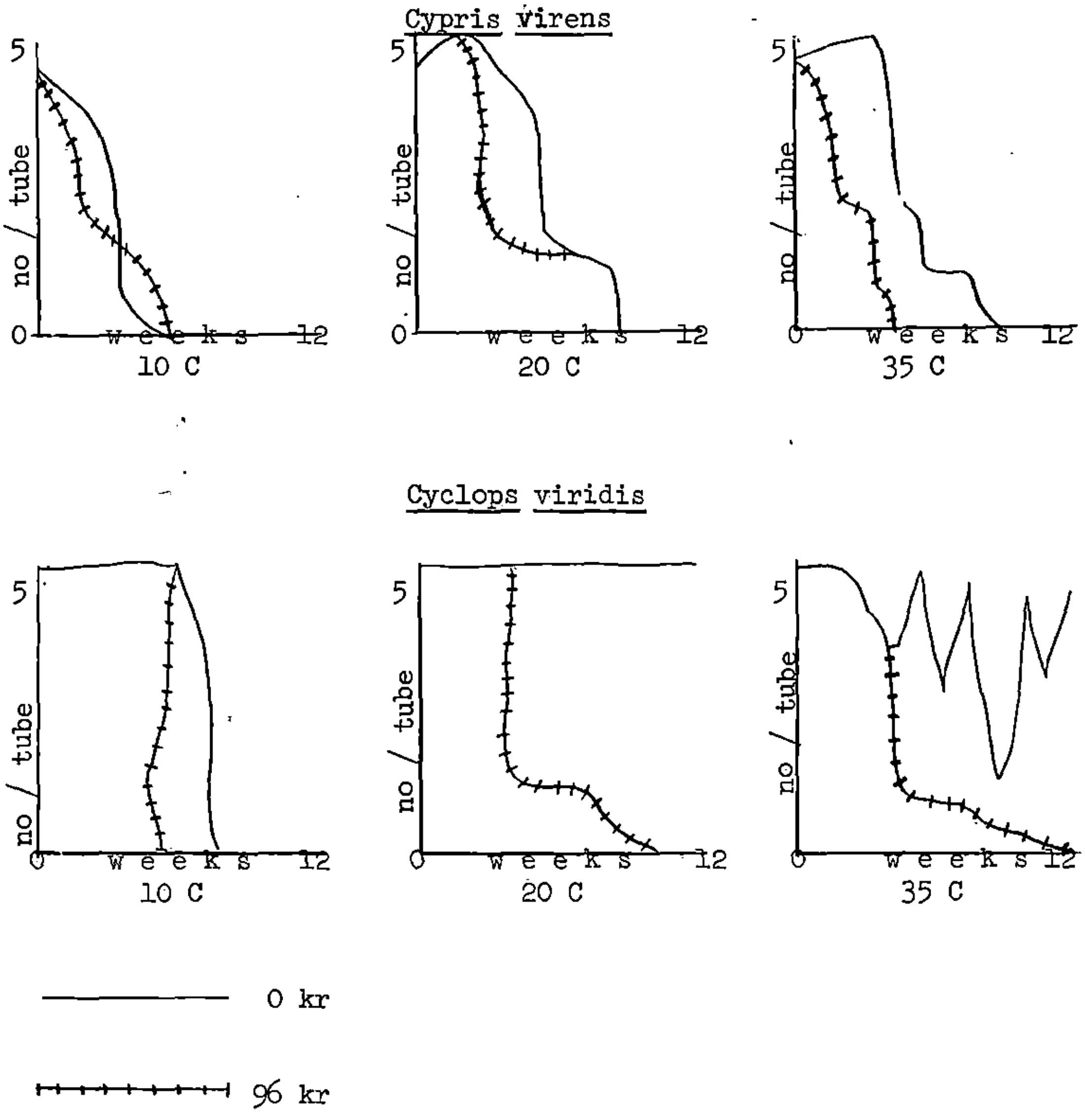


TABLE VI

Net Production of *Cypris virens* Exposed to
0, 24, 48 or 96 kr of Gamma Rays and Cultured at 10 C

No. of Weeks	IRRADIATION LEVEL IN KR					
	0	24	0	48	0	96
1	1000/ml	1000/ml	1200/ml	1200/ml	1600/ml	1600/ml
2	2200/ml	850/ml	2800/ml	750/ml	1650/ml	925/ml
3	2500/ml	500/ml	2500/ml	500/ml	1300/ml	750/ml
4	2100/ml	450/ml	3000/ml	400/ml	1700/ml	250/ml
5	2000/ml	400/ml	3000/ml	400/ml	1700/ml	250/ml
6	1800/ml	300/ml	2700/ml	350/ml	1500/ml	250/ml
7	1600/ml	200/ml	2400/ml	225/ml	1200/ml	150/ml

FIGURE X

Net Production of *Cypris virens* Exposed to 0, 24, 48 or 96 kr
Of Gamma Rays and Cultured at 20 C

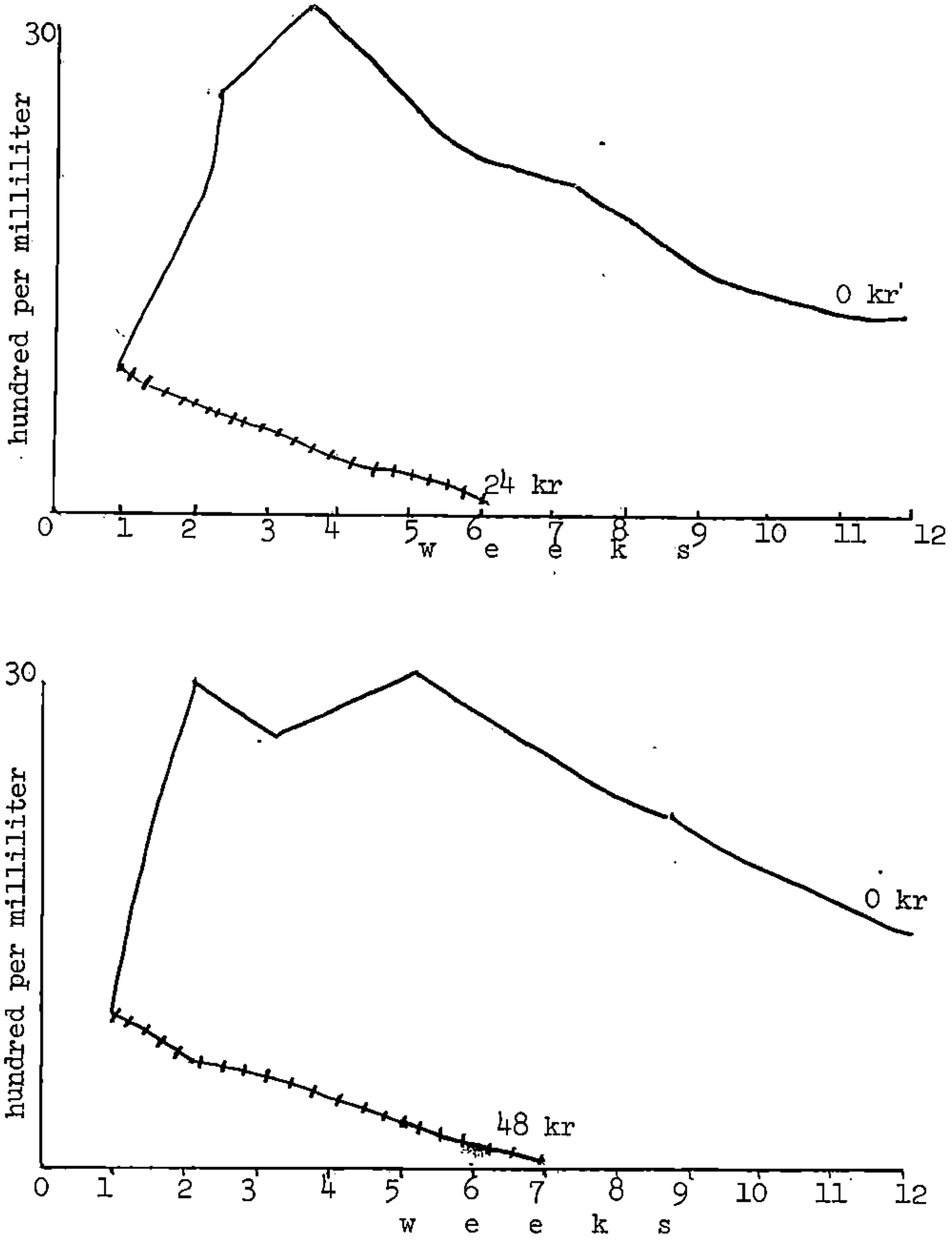


FIGURE X (continued)

Net Production of *Cypris virens* Exposed to 0, 24, 48 or 96 kr
Of Gamma Rays and Cultured at 20 C

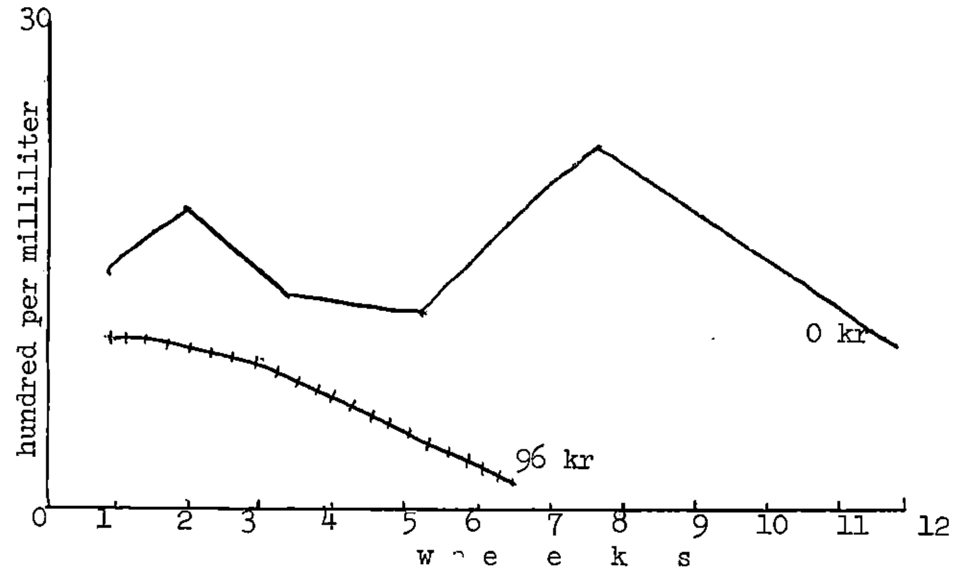


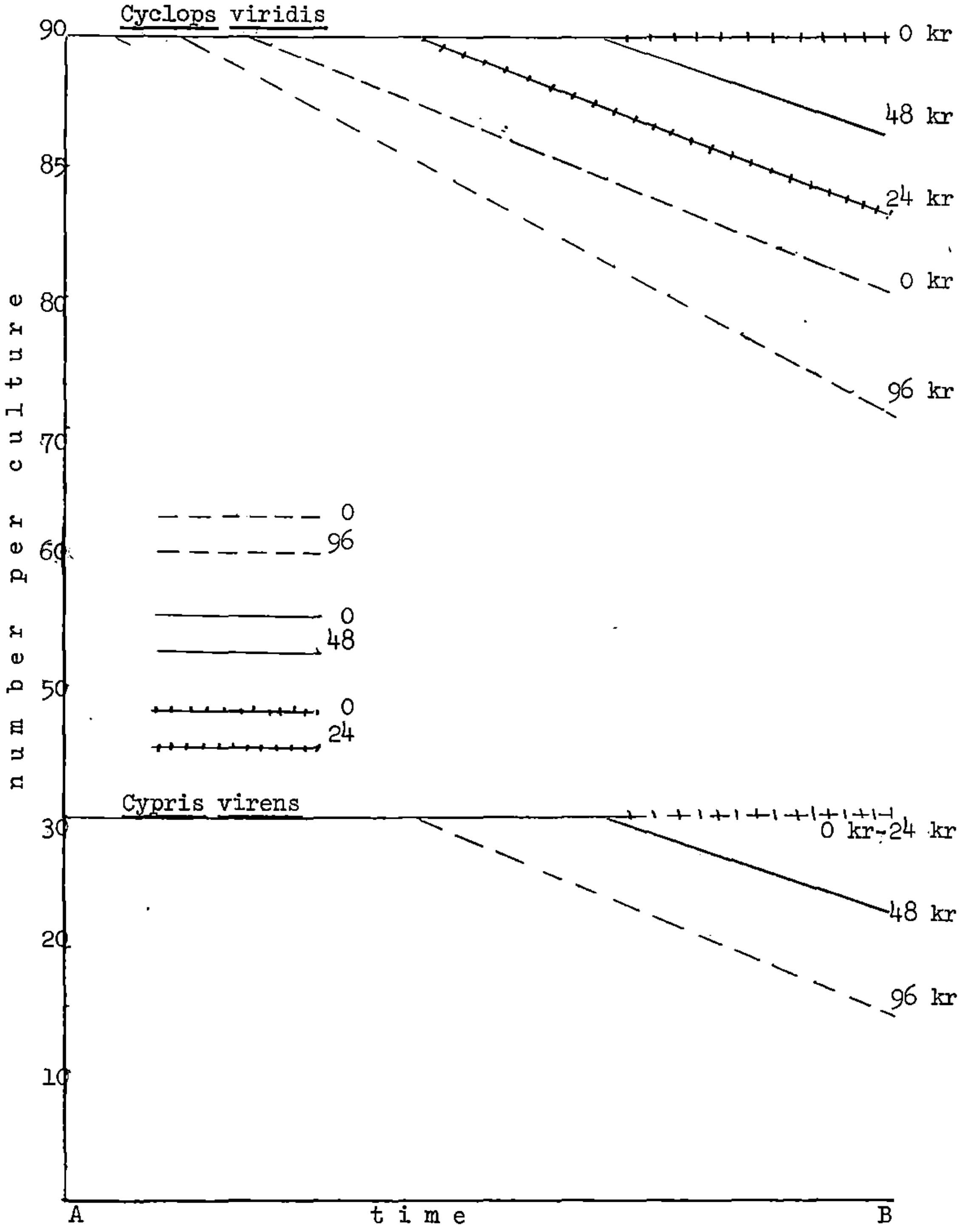
TABLE VII

Number of Adult *Cyclops viridis* and *Cypris virens* Per Culture Preceeding (A) and Following (B) Exposure to 0, 24, 48, and 96 kr Of Gamma Rays

Radiation Level	Organism	A	B
0 kr	Cyclops viridis	90	90
	Cypris virens	30	30
24 kr	Cyclops viridis	90	86
	Cypris virens	30	30
0 kr	Cyclops viridis	90	90
	Cypris virens	30	30
48 kr	Cyclops viridis	90	88
	Cypris virens	30	25
0 kr	Cyclops viridis	90	80
	Cypris virens	30	30
96 kr	Cyclops viridis	90	75
	Cypris virens	30	17

FIGURE XI

Number of Adult Cyclops viridis and Cypris virens Per Culture Preceeding (A) and Following (B) Exposure to 0, 24, 48, and 96 kr of Gamma Rays



DISCUSSION

The preliminary portion of this research dealt with the effects of temperature stress on the adult population density of cyclops, Cyclops viridis, and an ostracod, Cypris virens, cultured separately and together. Adult population density of cyclops cultured alone remained constant at both the 10 and 20 C temperature regimes. At the close of the twelve week experimental period, there were the same number of cyclops as were put in initially (Table I). The adult cyclops population cultured at 35 C decreased steadily until the density was 0 at the end of the fifth week (Figure I). The adult ostracod population cultured alone also remained constant at the 10 C level; however, the population cultured at 20 C remained constant for 10 weeks and then steadily decreased to 0 by the end of the 12 weeks. The adult ostracod population cultured at the 35 C level decreased steadily to 0 in 8 weeks (Fig. I). Although the adult ostracod population decreased to 0, there were numerous immature stages present. The density of the adult cyclops population in the same culture with the ostracod population never varied more than one adult per tube for the 10 and 20 C regimes. At the end of the twelve week period adult population density for cyclops at 10 C and 20 C was 15 per culture (Fig.I). Adult cyclops population density at the 35 C level decreased to 0 by the end of the 12th week (Table III). High temperatures (35 C) are critical for adult cyclops survival in both pure and mixed cultures. The adult ostracod population density in mixed cultures decreased steadily at 10, 20 and 35 C.

The ostracod adult population became 0 in the 12 weeks at 10 C, in the seventh week at 20 C, and in 8 weeks at 35 C. Dewey(12), working with effects of heterogeneous population on adult density found that the more heterogeneous a population, the less the effect of adverse conditions. Monakov(32), working with the effects of temperature on fresh water invertebrates, found that organisms at colder temperature had greater life span duration and greater population density than those at higher temperatures. Results similar to Dewey's and Monakov's were found by this author.

After determining the effects of temperature stress on adult population density of pure and mixed cultures of cyclops and ostracod, the effects of varying temperatures on both net production (immature stages) and adult population density were investigated for mixed cultures of the experimental organisms.

The total net production (immature stages) of both cyclops and ostracod cultured together at 10 C was greater than production at either 20 or 35 C. During the 12 week experimental period, the net production of cyclops decreased from 30 to 8 per culture tube and ostracod production increased from 1600 to 4000 per ml at 10 C; the net production decreased from 15 to 2 cyclops per culture tube and from 1200 to 900 ostracod per ml at 25 C; the net production decreased from 10 to 1 cyclops per tube and from 500 to 225 ostracod per ml at 35 C (Table II). Similarly, Monakov found that total net production, as well as life span, was greater at the colder temperature regime, as compared to higher levels.

Adult population density of both cyclops and ostracod cultured together was higher at 10 C than at either 20 C or 35 C. The adult cyclops population density increased from five to eight per tube and ostracod density decreased to 0 at the end of the eleventh week. Life span duration of adult ostracod was greatest at 10 C. Population density of cyclops was greater at the 20 C regime than at 35 C. The density for cyclops at 20 C decreased from five to three per tube and to 0 at 35 C at the end of the eleventh week. Ostracod population density and life span duration was greater at 35 C than at 20 C. Although the ostracod densities varied slightly at 35 C, their life span increased by two weeks. It was also noted that body size and fertility for both cyclops and ostracod was greater at 10 C than at either 20 or 35 C. The male cyclops proved to be more resistant to temperature than the female; however, females with eggs were more sensitive to temperature variations than adult males. McLaren(29), working with the effects of temperature on production of aquatic invertebrates, determined that adult population density was greater at the colder temperatures than at the higher levels. Spraque(41) found that cyclops reproduction was greater at colder temperatures, as was body size when compared to higher levels. Spraque also found male cyclops to be more resistant to temperature variation than females(6). My results confirmed both McLaren and Spraque.

After determining the effect of temperature stress on mixed cultures of cyclops and ostracod, modification of the effects by radiation were investigated.

The total net production of irradiated mixed cyclops and ostracod popultaions cultured at 10 C was greater than the production at either 20 or 35 C. The organisms exposed to 24, 48, and 96 kr of gamma rays and cultured at 10 C had higher production densities than the control organisms. There was an increase in net production at 10 C over the 12 week period. The total net production at 10 C was 3600 per ml at each level of irradiation. The net production for the irradiated organisms at 20 C was slightly higher than the net production at 35 C. The control cultures at both 20 and 35 C had much higher net production for the irradiated organisms. The net production of the irradiated organisms in the 12 week period decreased from 1600 to 1000 per ml at 30 C and from 1900 to 800 per ml at 20 C (Fig. IV, V, VI; Table IV).

Adult mixed population density for both irradiated cyclops and ostracod was greater at the 10 C level than the density at 20 or 35 C. Hence, life span duration was shortened by higher temperatures. Irradiated cyclops adult population density remained the same at 10 C and 20 C for a 12 week period, but decreased to 0 at 35 C in 4 to 5 weeks. At a given temperature the adult population density of both species was the same for all irradiated levels. Consequently, temperature rather than irradiation level is the critical factor in adult population density and life span (Fig. VII, VIII, IX; Table V). At 24, 48, and 96 kr all adult organisms died before 12 weeks. The adult cyclops population decreased to 0 in 8 to 10 weeks at 10 C, 6 to 8 weeks at 20 C and 4 to 5 weeks at 35 C. At control conditions (0 kr irradiation) cyclops at 10 and 20 C continued to live; at 35 C all cyclops died within the experimental period. The control adult ostracod population density and life span was 2 to 3 weeks longer than life span duration and population density for irradiated organisms.

Beyers(5) found that the lower the temperature the lower the rate of metabolism; and Ghys(1) concluded the lower the rate of metabolism, the less the effect of radiation. Thus, one might expect a greater adult population density and greater net production at the 10 C level at any exposure of irradiation up to 100% lethality than at the higher temperatures tested. Although there was no reproduction of cyclops following exposure to 24, 48, and 96 kr of gamma rays, life span and population size were greater in all populations cultured at 10 C than at 20 or 35 C.

Marshall(27, 28) found that fertility was the most evident effect of irradiation of fresh water crustacea with life span shortening and adult mortality being secondary. My results confirm these findings. Combined effects of irradiation and temperature stress resulted in a decreased life span at 35 C and an increased life span at 10 C over the life span at 20 C for all levels of irradiation tested.

Net production of ostracods not exposed to irradiation and cultured at 20 C showed a gradual decrease and leveled off at 1200/ml in the seventh week. At the exposure level of 24, 48, and 96 kr of gamma rays, a decrease in net production to 400 per hundred ml at the end of the seventh week occurred (Table VI). With the exception of slight adult cyclops mortality immediately following gamma ray exposure at 96 kr of gamma rays, the adult survival was the same at all levels of irradiation. However, the ostracod adult mortality did vary immediately after irradiation (Table VII). The survival was decreased 15% after exposure to 24 kr; 23% after exposure to 48 kr, and 47% after exposure to 96 kr. Marshall, in 1965, working with adult daphnia populations, found that radiation exposure levels below LD 50 had little to no effect on adult mortality and that net production of irradiated daphnia at 20 C was not appreciably effected by radiation exposure levels. Results of this study confirm Marshall.

SUMMARY

The effects of radiation and temperature stress on an aquatic ecosystem comprised of Cyclops viridis and Cypris virens were evaluated. Adult population density studies were performed on experimental species cultured both together and separately. Total net production (numbers of immature stages) and adult population density were determined for a mixed culture of cyclops and an ostracod at three temperature regimes. After determining the effect of temperature stress on mixed cultures of the experimental species, modifications of the effects by irradiation were investigated. A study was performed on adult survival immediately following gamma ray exposure of mixed cultures of irradiated cyclops and ostracods. The results showed the following.

(1) Adult ostracods cultured in the absence of cyclops had a longer life span and greater population density at all three temperature levels than those in the mixed cultures. Cyclops showed a greater adult population density when cultured with ostracods.

(2) Total net production and adult population density for both experimental organisms were greater at 10 C than at 20 C or 35 C. Life span duration for both species was longer at 10 C than at either 20 C or 35 C.

(3) Adult survival of cyclops was unaffected immediately after radiation exposure at both 24 and 48 kr levels. The level critical to cyclops reproduction is between 8 and 16 kr. Ostracod survival immediately after irradiation decreased continually as the gamma ray exposure increased.

(4) Adult population density and net production for both organisms varied insignificantly between 24, 48, and 96 kr at 20 C. At 10 C net production, life span, and adult population were considerably greater than net production and population density at either 20 C or 35 C at all levels of irradiation. The net production, life span, and population density of the irradiated organisms cultured at 35 C was considerably lower than those at 30 C or 10 C.

(5) Net production of irradiated organisms was significantly lower than controls; however, net production of irradiated organisms varied very little between radiation levels of 24, 48, and 96 kr.

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