

AN ABSTRACT OF THE THESIS OF

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Title: EFFECTS OF ^{60}Co GAMMA IRRADIATION ON THE REPRO-
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The brine shrimp, Artemia, was used as an experimental organism to study the effects of ^{60}Co gamma irradiation on the reproductive performance of an animal population. The total reproductive ability of the brine shrimp was fractionated into various components and the effects of irradiation on each of these components was then determined by studies of reproductive behavior in individual pair matings. In this study, the components identified were the number of broods produced per pair, the number of nauplii voided per pair, the number of nauplii voided per brood, the survival of nauplii to sexual maturity, the number of mature adults produced per brood, and finally the number of mature adults produced per pair.

All component parameters of total reproductive performance were shown to be affected by irradiation. However, the number of broods per pair was shown to be the factor most affected by doses

of 1200 rads or less.

The final parameter, the number of mature adults produced per pair, is really the measurement of the net reproductive potential of Artemia. The net reproduction was also examined by making counts of the total population contained in three liter population cultures, and contrasted to the results obtained with the pair mating studies.

It was demonstrated that the population cultures may be maintained by using only a small part of the reproductive potential exhibited in the pair matings. Therefore, we find that the results of pair matings must necessarily be used to assess the amount that the reproductive potential of Artemia is decreased due to various doses of irradiation.

It was determined that for Artemia irradiated at the most sensitive stage, a dose of 2100 rads produced sterility. At less sensitive stages, more than 3000 rads would be required to produce sterility.

In a single experiment, Artemia irradiated with 300 rads gave an indication of a slight enhancement of reproductive ability as compared to the control animals. In all cases, doses of 600 rads or less showed little effect on the reproductive ability of this species.

Effects of ^{60}Co Gamma Irradiation on the Reproductive
Performance of the Brine Shrimp, Artemia

by

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THE EFFECTS OF ^{60}Co GAMMA IRRADIATION ON THE
REPRODUCTIVE PERFORMANCE OF THE
BRINE SHRIMP, ARTEMIA

INTRODUCTION

There is a sizable amount of literature which is concerned with the effects of ionizing radiation on physiological processes as well as with genetic change and the subsequent appearance of mutations in future generations. The recent publication by Grosch (1965) summarizes much of this research. The emphasis in the present study is different. In this case, the irradiation is considered as an ecological factor and its effect on the reproductive performance of a laboratory population is evaluated. Only by successful reproduction can a species maintain its place in the ecosystem. A second difference in this study, even as compared to some other ecologically oriented studies (Park, DeBruyn, and Bond, 1958), is the study of the effect of doses as low as 300 rads on the reproductive ability of an animal population, since larger doses have less significance for population maintenance under current ecological conditions.

The population is one of the fundamental levels of biological organization. It is the functioning unit of evolution (Mayr, 1963) and it is also a basic structural unit which combines with populations of other species to form the community (Odum, 1959). Since the

population is a fundamental structural component of the ecosystem, the study of the response of a population to radiation stress is essential to a sound understanding of radioecology. Our interest in the effects of radionuclide contamination of the environment upon the ecosystem can be furthered by an investigation of the reproductive performance of the component populations when they are subjected to radiation stress.

In a study of the radiosensitivity of the ecosystem, the reproductive ability of each population that is important in the bioenergetics of an ecosystem must be evaluated, since the stability of the ecosystem as a whole may depend upon the ability of its most radiosensitive population to maintain a normal population size in the event of radionuclide contamination. If any population in the ecosystem is reduced or eliminated by radionuclide contamination, populations at both higher and lower trophic levels will be affected indirectly, even though they were little affected by the radiation itself, and the balance of the ecosystem may be destroyed (MacArthur and Connell, 1966).

This study was initiated to add to our understanding of the effects of various doses of gamma irradiation upon the reproductive ability of an animal population. Such knowledge is essential if we are to understand the effects of present and future doses of irradiation upon the various populations and hence on the production and evolution of various ecosystems of our modern world. However, in the

environment the situation is far from this simple. We must not only know the effects of these various doses of irradiation upon the reproductive ability and physiological functioning of the population, but we must also know the degree to which a particular plant or animal species concentrates the various radionuclides, where they are located in the organism, and what dose these nuclides will deliver to the germinal tissue as well as to important somatic tissues which might affect the physiological functioning of the organism in a competitive situation. Finally we must consider the other effects of incorporated radionuclides, besides the effects of the ionizing irradiation that they produce. If a particular radionuclide is incorporated in a molecule of biological importance, the effect of the recoil energy imparted to the nucleus or the changes in chemical bonding resulting from the transmutation from one element to another may be potentially far more harmful than the ionizing irradiation produced. Stent and Fuerst (1955) have shown for example, that the relative importance of transmutation is much greater than the effects of ionizing irradiation for inactivation of the phage cultured with ^{32}P in the media.

A series of experiments have been performed to evaluate the effects of acute doses of gamma rays, from a ^{60}Co source, upon the reproductive ability and hence on population maintenance of laboratory populations of the brine shrimp, Artemia. The brine shrimp was chosen as the experimental organism for several reasons. First,

it was considered desirable to extend population radiosensitivity studies to a crustacean. Second, the brine shrimp proved to be a very tractable laboratory animal that could easily be reared through many successive generations. Finally, the attention was focused on this animal by the work of Grosch (1962, 1966) in which he studied the effects of the accumulation of the radionuclides ^{65}Zn and ^{32}P upon the reproductive behavior of Artemia. This research allows some comparison between the effects of incorporated radionuclides and the effects of irradiation from an external source.

Grosch (1962, 1966) has demonstrated the effects of the addition of ^{65}Zn and ^{32}P to three liter population cultures of Artemia. He has concluded that although the number of adults counted in population cultures may be the same, the animals from experimental cultures have a shortened life span, deposit fewer zygotes per brood, and show poor survival to adulthood as compared to control animals which have not been subjected to radionuclide contamination. He also has determined that population cultures of control animals use only 0.2 percent of the reproductive potential that they exhibit in pair matings to maintain the population size. However, populations of experimental animals are required to use one percent or more of their potential to maintain the same population size. Hence, although we may not detect the effects of radioactive contamination when studying laboratory population cultures, such effects could be

manifest in a competitive environment.

Of particular interest, however, are Grosch's (1966) results which show that the addition of as little as 20 μCi of ^{65}Zn to a three liter population culture of Artemia has consistently caused the cultures to become extinct. In the case of ^{32}P , the populations are able to stand the addition of greater amounts of activity. Cultures routinely survive the addition of an aliquot of 30 μCi ^{32}P per three liter culture, and one culture has survived the addition of as much as 90 μCi ^{32}P .

At least a part of the explanation for this different response to the two nuclides may be in terms of the fact that the ^{65}Zn has a 245 day physical half-life and the ^{32}P has only a 14 day half-life. A culture receiving the addition of 20 μCi of ^{65}Zn would be exposed to a higher level of activity over a longer period of time than a culture receiving 20 μCi of ^{32}P . Therefore, such a ^{65}Zn culture would be exposed, during the course of the experiment, to many more radioactive decay events than a ^{32}P culture which received the same initial dose of activity. Other differences in the decay of ^{32}P and ^{65}Zn must also be considered in any discussion of the results of Grosch (1966).

The decay of ^{32}P proceeds by the emission of a single negatron with a maximum energy of 1.71 Mev and a mean energy of 0.70 Mev. The decay of ^{65}Zn is more complex, with electron capture

followed by the emission of a 1.11 Mev gamma ray accounting for about one half of the decays. Another 49 percent of the decays are accomplished by electron capture alone, while 1.7 percent of the time the decay is accomplished by emission of a positron (Wang and Willis, 1965). Hence, in the case of ^{65}Zn , the energy to produce ionizations in living tissue may come from the gamma ray emitted, from x-rays which are produced during the rearrangement of orbital electrons following electron capture, from interactions with the positron or finally from the gamma rays produced upon annihilation of the positron.

However, in this study we are interested in determining what part the ionization in living tissue plays in the observed effects, as compared to the effects due to transmutation and also due to the recoil energy of the decaying nucleus. The present study, using an external source of gamma rays from ^{60}Co , will allow for some degree of assessment of the effects of certain selected doses of ionizing radiation on population maintenance of the brine shrimp, without having to consider transmutation and recoil effects as potential sources of error in the interpreting of the results.

In particular, this series of experiments was designed to establish dosage levels, in rads, at which the reproductive ability of Artemia was affected and to determine the dose required to reduce the reproductive ability of the population to zero and thus fix the

level at which a laboratory population would be unable to reproduce itself and hence go to extinction.

METHODS AND MATERIALS

The Organism

The genus Artemia is a member of the Order Anostraca, Subclass Branchiopoda, Class Crustacea of the Phylum Arthropoda (Barnes, 1963). Each of the members of the Branchiopoda has an epipodite on the thoracic appendages which serves as a gill, hence the name Branchiopoda, meaning "gill feet." The Anostraca are called "fairy shrimp." They have no carapace and the compound eyes are stalked. Other members of the order typically inhabit temporary fresh water ponds, while the genus Artemia is found in both temporary and permanent ponds of high salinity.

Although laboratory experiments show that Artemia survives and reproduces for many generations in sea water, it is never found in this environment in nature. However, it is found widely distributed in more saline waters in many parts of the world. It is apparently able to survive only where members of higher trophic levels are eliminated by the increased ionic concentration of these waters (Carpelan, 1957).

The taxonomy within the genus Artemia is confused. Originally old world populations were called Artemia salina Leach, and we find that this name is often still applied to all members of the

genus. However, the taxonomy of the genus has had an interesting history. A survey of the old world taxonomy (Stella, 1933) shows that as different morphological types were recognized in the old world, the genus was split into two species based on the structure of the caudal furca. In North America the name A. salina is also often used to grossly describe all members of the genus. However, here too as morphological characters were noted, taxonomists tended to split up the genus into many different species. For example, Kellogg (1906) lists four North American species: A. gracilis Verrill, described from near New Haven, Connecticut; A. fertilis Verrill, from Great Salt Lake, Utah; A. monica Verrill, from Mono Lake, California, and a new species, A. franciscanus Kellogg, from evaporating pools at Redwood City, California. However, Pratt (1916) compounded the problem by using A. gracilis to describe the brine shrimp of the Great Salt Lake. This name has been used commonly by other authors (Jensen, 1918; Relyea, 1937). However, in the early decades of this century, research was carried out which showed that much or all of the observed morphological differences noted in different populations was due to the concentrations of salt in which the species was growing (Boone and Bass-Becking, 1931; Stella, 1933). Eggs from different populations when reared under standard conditions, were shown to produce very similar phenotypes.

As the importance of this work was assimilated, it became

common practice to again refer to all members of the genus as Artemia salina (Pratt, 1935; Bond, 1937; Lochhead, 1950; Barigozzi, 1957; Bowen, 1962). However, the question arises as to what type of a criteria should be used to define the relationships between the various populations within the genus. If we accept the concept of the existence of reproductive isolation (Mayr, 1963) between populations as sufficient cause to define two populations as two species, it is apparent from the work of Bowen (1964) that the genus consists of at least three species based on her study of only nine populations. Certainly the tractibility of this genus in the laboratory and the large number of populations distributed world-wide provides an excellent opportunity for the study of the taxonomy of this genus.

Due to the taxonomic problems cited, we will refer to the organism used by the generic name Artemia in this paper. However, the particular organisms used were from the population which inhabits the Great Salt Lake, Utah, in the United States. The particular cysts used were collected in August of 1965 by the Brine Shrimp Sales Company of Hayward, California. All experimental work reported was conducted between September, 1966 and June of 1967, hence the cysts were between one and two years of age during the course of this work.

This population is a diploid amphigonic population (Bowen, 1964) which is well known due to its extreme abundance in

Great Salt Lake. Cysts are harvested regularly and sold commercially to be hatched as food for aquarium fishes.

The Life Cycle

It will be possible to discuss the reproductive performance of this organism only after a rather complete review of the life cycle for Artemia. For this discussion, only the diploid amphigonic life cycle of the population used in these experiments will be considered. The resting "egg" of the brine shrimp is itself unusual. This structure might be better spoken of as a cyst since it is not an egg, but is typically an embryo in which the development is arrested at the blastula stage (Bowen, 1962). This blastula is covered with a thick brown proteinaceous covering which may protect the embryo for as long as 15 years before hatching (Lochhead, 1941). However, under favorable environmental conditions of a temperature of at least 10° C and in less saline water the eggs appear to hatch readily (Jensen, 1918).

As we will see later, this resting cyst is only one of three types of reproductive "eggs" common in the genus. It is also an excellent example of the adaptation of an organism to its environment. The heavy shell of this cyst is not excreted unless some environmental stress is placed on the animal. Bowen (1962) states that these cysts never appear in laboratory cultures maintained under

optimum conditions, and observations during the course of the experiments reported here support this conclusion. However, in cases of decreasing temperatures as winter approaches or in cases of extremely high salinity in evaporating basins used for commercial salt production, the resting cysts are produced (Jensen, 1918; Carpelan, 1957). The hatching of the cysts will occur much more readily in waters of lowered salinity, as for example in the spring with increased fresh water appearing in the saline lakes and basins.

Heath (1924) gives us a detailed account of the embryology of Artemia. His specimens were collected from the salterns around Redwood City, California. He states that his specimens are Artemia salina var. principalis Simon, but no other information is available on this population. However, it is noted from his measurements that his Artemia had mature males (12th to 13th instar), about 6.5 to 7.0 mm in length. This is smaller than those listed by other authors. Jensen (1918) listed the males as from 8-10 mm long, and mature males from the population used for these experiments were from 10-12 mm long.

The development consists of a series of 11 molts, hence 12 instars for the shrimp to reach sexual maturity. Upon reaching adulthood, the shrimp continues to molt every four to six days (Bowen, 1962).

Upon hatching, this genus emerges as a typical nauplius bearing the customary three pairs of appendages. In successive

instars we observe the development of the thoracic appendages, modification of the body shape and specialization of the head appendages. In particular at about the 8th instar we can detect a sexual dimorphism manifest in the structure of the second antennae. In the female the second antennae which were used for locomotion in earlier stages are reduced greatly in size. In the male the structure of the second antennae is increased in size and modified in structure to be used to grasp the female during copulation. By the 9th instar the modifications are distinct and the sexes easily distinguishable.

It should be mentioned that the 11 pairs of thoracic appendages serve a dual purpose. They are used for locomotion and enable the animal to move from the surface to the bottom of the typical shallow lakes and ponds that they inhabit. Hence they take planktonic algae or littoral forms as the situation demands. However, the ability of locomotion is slight and if subjected to water currents, the brine shrimp would certainly be swept along as part of the plankton. These appendages also serve in food-getting as well as in locomotion. Cannon (1933) has shown that the thoracic appendages set up a current of water which brings food into the ventral food groove, where cilia and the mouth parts are used to carry the food the rest of the way to the mouth. This mechanism is apparently very efficient as Lochhead (1941) reports that the brine shrimp can retain particles as small as $1/4 \mu$.

After the male attains sexual maturity, he grasps the female during copulation, with his enormous specialized second antennae. The female violently resists this action, but a successful male secures a position dorsal to and partly behind the female, with his antennae locked around her body just in front of the ovisac. The two animals are firmly united and swim about in this fashion. At intervals the male curls forward the hind part of his body, attempting to insert one of the two male organs into the uterus. The female does not permit this unless she has recently completed a molt, successful copulation being restricted to this period.

The reproductive system of the female consists of two ovaries, two oviducts and a ventral uterus. The following sequence of events occurs in the adult female (Bowen, 1962). The female expels from the uterus an egg generation, the process taking two to ten hours. Then she molts in a few seconds and the next egg generation passes from the ovaries to the oviducts in less than two hours. Copulation appears to be effective only while the eggs remain in the oviducts with fertilization occurring as they pass from the oviducts into the uterus. Shortly after the eggs enter the uterus, the shell glands may begin to secrete the dark shell material which surrounds the cysts.

Lochhead (1941) and Bowen (1962) have shown that under ideal conditions, the shells are not formed and the cysts hatch to yield free living nauplii before they are expelled from the uterus. Hence

we have a viviparous type of birth. However, in some cases, a thin shell is formed and the cysts which are expelled typically hatch in about one day. These cysts are often called "summer eggs." A third possibility is the formation of the thick shelled "winter eggs," which are capable of surviving for a long time under adverse conditions. As stated before, these eggs seem to be produced only in cases of environmental stress.

Cytological studies (Goldschmidt, 1952) have established the fact that while the eggs are in the oviduct, they are in the metaphase stage of the first meiotic division. It appears that fertilization is a necessary stimulus for the completion of meiosis. This fact is especially useful for studying the state of ploidy and chromosome morphology in the genus. Using this fact to advantage has led to the discovery of various polyploid populations within the genus (Barigozzi, 1957).

Culture Methods

During the year preceding the experimental work, brine shrimp were maintained in culture in pilot studies to determine proper conditions for the growth and reproduction of the animals. At the same time, techniques were developed for handling the cultures during irradiation and for counting the animals in the cultures by methods which reduced damage from handling to a minimum. In one

pilot study, cultures of shrimp counted each day for ten days showed no significant increase in mortality, as determined by a X^2 test, when compared to cultures handled and counted only at the beginning and end of the ten day period. The results of this experiment are presented in Table 1. The following culture and handling procedures are based on these pilot studies and these culture procedures were followed during the entire course of the experimental work.

Table 1. Effect of daily handling on the survival of young adult Artemia.

	Culture Number	Original Number	Number on Tenth Day	Total
Control cultures	1	20	19	
(Handled and counted only on first and tenth day)	2	20	16	
	3	20	20	
	4	20	19	
	5	20	19	93
Experimental cultures	6	20	15	
(Handled and counted each day for ten days)	7	20	19	
	8	20	17	
	9	20	20	
	10	20	18	89

$$\begin{aligned}
 X^2 &= \sum \frac{(|O - E| - .5)^2}{E} = \frac{(|93 - 91| - .5)^2}{91} + \frac{(|89 - 91| - .5)^2}{91} = \\
 &= \frac{1.5^2}{91} + \frac{1.5^2}{91} = \frac{2.25}{91} + \frac{2.25}{91} = .0247 + .0247 = .049
 \end{aligned}$$

$$X^2_{.05} = 3.84$$

All counts of sizes of both population cultures and production of pair matings were made by straining the culture through a 1100 μ

silk bolting cloth. The adults were retained on the cloth with younger stages and feces being washed through. The adults were back washed off the cloth and distributed upon a glass plate in groups of a few animals in a drop of culture water. In this way a sizable population was rapidly counted and returned to the culture container in a short period of time. Counts of nauplii were made by straining the culture through a synthetic fiber filter paper and back washing and proceeding as with the adults above. The only pipetting of the animals was during removal of adults that had reproduced from their brood to a fresh culture container. The pipettes used for this work had a four mm minimum inside diameter to minimize the chance of damage during transfer.

All cultures were maintained under constant illumination in the laboratory. The laboratory used had no outside windows, and it was found that the animals reacted to the sudden turning on of the room lights after being in total darkness by several minutes of very rapid random movement before settling down to a normal swimming pattern typically exhibited in the light. During the course of the experiments, the temperature of the laboratory was maintained within the range of $21.0 \pm 0.7^{\circ}$ C.

Since all experimental work in this study was conducted using Artemia cysts from Great Salt Lake and since in their normal environment, this population is subjected to salt concentrations of

several times that of sea water, the question of the proper salt concentration to use in a laboratory study arose. A small experiment was designed to help resolve this question. Several groups of animals were reared in sea water and in sea water with various amounts of sodium chloride added, of from 50 to 150 g per liter. When mature, the shrimp were irradiated at several different doses and their survival was evaluated. The data is summarized in Table 2 and graphed in Figure 1. Each survival value in Table 2 is calculated on the basis of a single sample of 50 young adult Artemia.

Table 2. The percent of young adults surviving for 25 days at various doses of irradiation in four different culture waters.

Culture Media	Dose		
	0 Rads	4000 Rads	25,000 Rads
Sea water	68	47	44
Sea water plus 50 g NaCl/l	92	92	76
Sea water plus 100 g NaCl/l	66	18	10
Sea water plus 150 g NaCl/l	54	10	20

It should be noted that at all levels of irradiation the survival was best in the sea water with 50 g of added sodium chloride per liter and this water was used as a standard culture water. It would seem likely that this culture water would most nearly approximate the

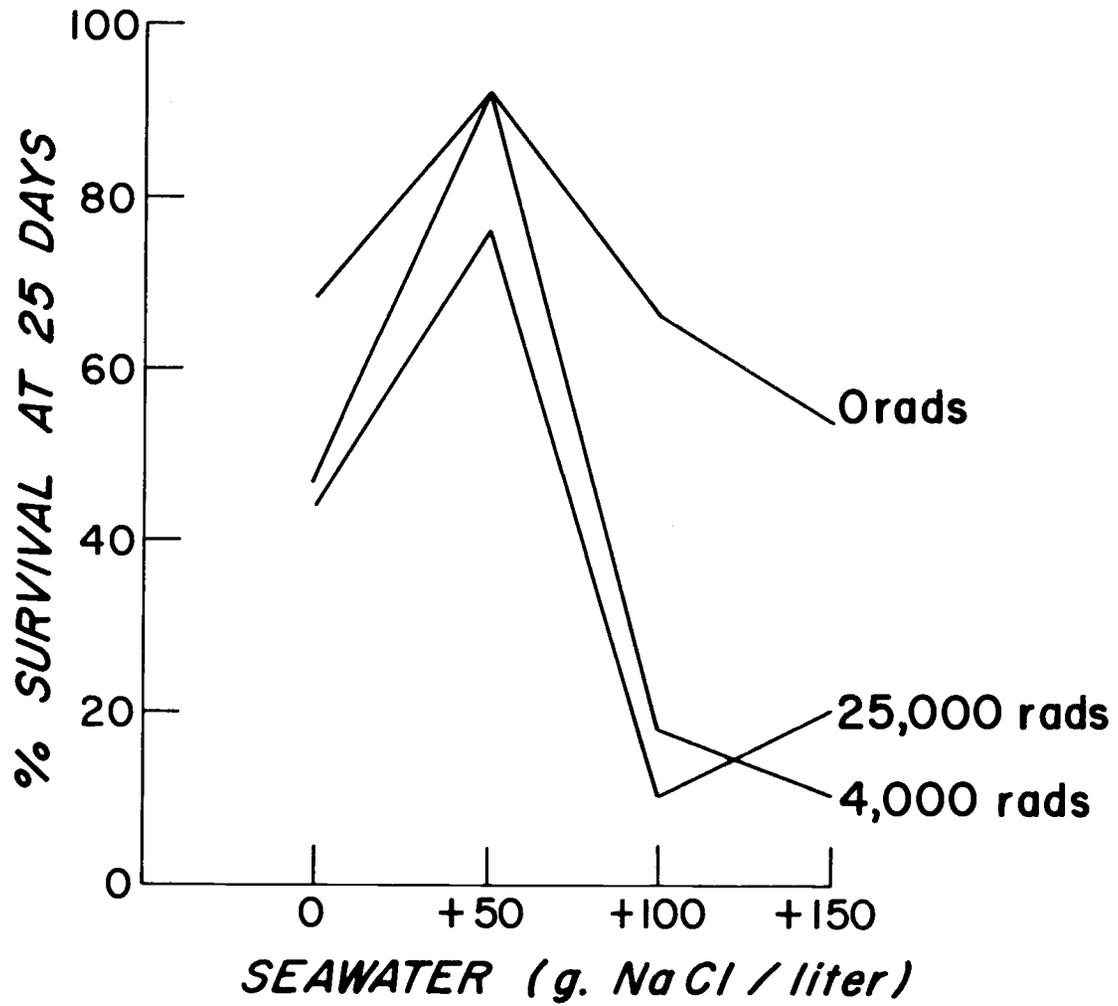


Figure 1. The percent of young adult *Artemia* surviving for 25 days at various doses or irradiation in four different culture waters.

environmental conditions of Great Salt Lake, in which this population evolved, of any of the various waters tested. It is interesting that both Bowen (1962) and Grosch (1966) have also adopted culture water with 50 g of NaCl added to each liter of sea water for their work with Artemia, although they used different criteria for selection of this culture water. At the time these experiments were performed, an effort was made to culture Artemia in distilled water with 35 g of sodium chloride added per liter. This would approximate the amount of total salts in sea water, but of course would not duplicate the sea water in ion composition. However, in the original experiment and in a replicate experiment later, the nauplii introduced into this water were able to survive for only three days, indicating the importance of some balance of various ions for survival of Artemia.

All cysts were hatched in sea water, with hatching being complete in 48 hours after hydration of the cysts. At 48 hours after hydration, the newly hatched nauplii were transferred with a large pipette to the standard culture water and remained in this water during the course of the experiments. The sea water for all work was purchased at the Aquarium in Depoe Bay, Oregon. Their intake for sea water is located just offshore in the North Pacific Ocean in a region isolated from fresh water runoff. This water was strained before storage through a prefilter and then through a 0.45 μ Millipore Filter. The water was then stored until needed in the dark, in a cold

room at 9° C. Under these conditions very little contamination with algae was encountered, with only about one culture in fifty developing a bloom of a very small unicellular green algae.

Animals were studied in both population cultures and by means of individual pair matings. The population cultures were set up in three liters of culture water and the size of the population determined at successive intervals by removing the animals from the culture. Sexing and counting the animals was followed by returning them to their original population culture. The preliminary experiments cited, as well as controls carried along during the principal experiments, indicate that this handling can be accomplished with no damage to the animals.

Individual pair matings were made in approximately 500 ml of culture water (one pint polyethylene freezer cartons). Each pair mating was examined daily and when young were noted, the parents were transferred into a new culture container and the young were raised to maturity in the original container. Nauplii were counted on the third day after they emerged, returned to the culture container and counted again and sexed at maturity.

All animals were fed daily a standardized suspension of brewer's yeast in distilled water. This was prepared by the method of Bowen (1962) by adding one cubic centimeter of yeast to nine ml of distilled water. A standard amount of this suspension was added

to each population culture daily, in most cases one ml of the suspension was used. The same suspension was used for the pair matings, but the amount fed varied daily for each culture, depending on the number and size of the animals present.

Preliminary experiments in this laboratory indicated that aeration of the cultures was not required, and hence the experimental cultures did not receive this treatment.

Preparation for Irradiation

All animals to be irradiated were hydrated, hatched and transferred to standard culture water according to the techniques previously described. To provide adequate room to raise a large number of animals, an eight gallon aquarium was used as a nursery container. During the course of development, the animals were observed daily for uniform growth. For the pair mating experiment, to be described later, it was necessary to start three different cultures in order to obtain one culture which had an adequate number of uniform-sized animals to be used in the experiment. The non-uniform growth obtained in the first two attempts may have been due to overcrowding, a lack of food, or to a combination of these factors.

When the animals in the aquarium had reached the proper stage of development, they were thoroughly mixed and removed from the aquarium with a dip net and placed in a one liter container.

Small numbers of the animals were removed from the container with a large pipette, placed in a series of drops on a glass plate and counted. After counting, they were rinsed off the glass plate into a clean 500 ml culture container until the required number of Artemia were placed in each of several such containers. The containers were then filled with the standard culture water.

At this time the various containers were numbered and assigned to the various treatments at random, with the aid of a table of random numbers (Li, 1957).

Immediately before irradiation, the animals were removed from the culture containers by carefully filtering the culture through a 1100 μ silk bolting cloth and backwashing the animals off of the bolting silk with 10 ml of fresh culture water directly into the polyethylene tubes used during irradiation.

After irradiation, the animals were again filtered out of the water used during irradiation and backwashed directly into their correct culture container.

Conditions of Irradiation

The ionizing radiation used in all experiments consisted of gamma rays (1.17 and 1.33 Mev) from ^{60}Co . The irradiator, which is shown in Figure 2, contains a 3600 curie source which is separated from the sample chamber by a lead concrete shield. The

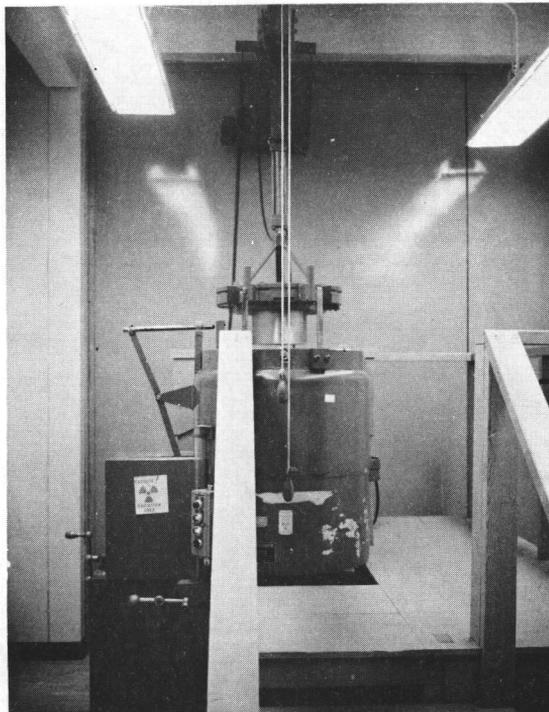


Figure 2. View of the ^{60}Co gamma irradiator located at the Radiation Center, Oregon State University, Corvallis, Oregon. The lid has already been lifted to expose the irradiation chamber.



Figure 3. Specially developed polyethylene holder and test tubes used to hold the Artemia while they were being irradiated.

source consists of 12 rods encased in stainless steel capsules which effectively remove all beta rays produced by the decay of ^{60}Co .

When the shield is drawn back, the 12 rods which are positioned in a circular arrangement, may be moved upward mechanically to surround the stainless steel irradiation chamber. The irradiation chamber is a cylinder 10-1/4 inches in diameter and 5 inches high with a high flux 5 inch x 5 inch circular well in the bottom. All irradiation was carried out in the high flux well.

The animals were irradiated in 10 ml of fresh culture water held in 50 ml polyethylene test tubes. Five of these test tubes could be irradiated at one time in the specially constructed polyethylene holder, shown in Figure 3 (page 24), which fits into the irradiation chamber as depicted in Figures 4 and 5. In the cases when not all of the five tubes were being used to irradiate animals, the unused tubes were placed in the tube holder with 10 ml of culture water in order to maintain a constant geometry of irradiation. During the course of the irradiation, the irradiation chamber was continuously perfused with air. This served to help maintain a constant temperature and to supply adequate oxygen to the animals, while also removing the toxic ozone which was produced in the air within the irradiation chamber. The temperature of the culture water was increased by less than 2° C by the longest irradiation time employed in these experiments.

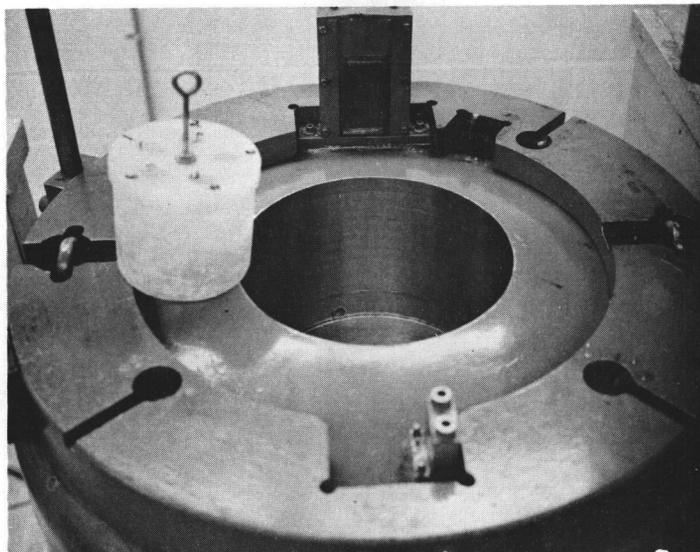


Figure 4. Polyethylene holder and tubes sitting at the edge of the opening to the irradiation chamber.

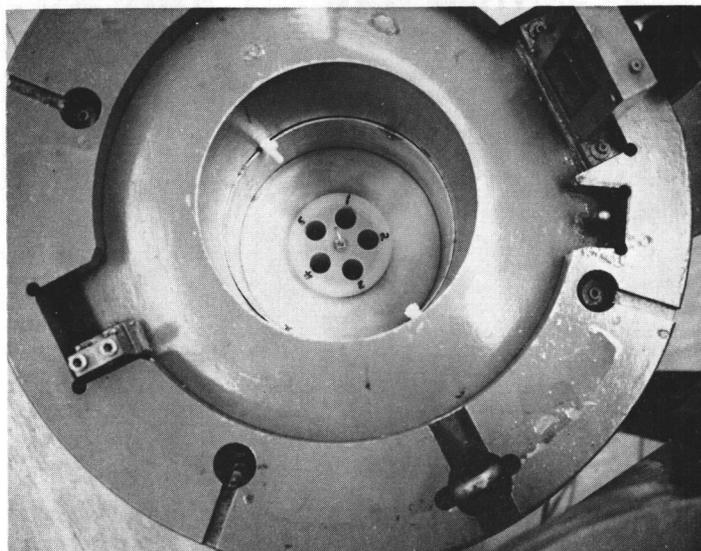


Figure 5. Polyethylene holder and test tubes in position, in the high flux chamber of irradiator.

In all experiments, parallel control cultures were maintained. These animals were subjected to the same handling as the irradiated animals and were placed in the irradiator for a time equal to the longest irradiation time used in that experiment. However, for the control cultures, the safety shield is not removed and hence they receive virtually no irradiation. In both of the major experiments to be reported on, a secondary control which was subjected to as little handling as possible was also maintained to help assess the extent of damage from handling. In both experiments, this secondary control showed that the handling during irradiation did not effect the survival of the Artemia.

Dosimetry

The dose rate delivered to the animals was determined by using the Fricke dosimeter, which consists of an aerated 10^{-3} M solution of ferrous ammonium sulfate, 10^{-3} M in sodium chloride and 0.8 N in sulfuric acid. The tubes were filled with 10 ml of the ferrous solution, irradiated, and the concentration of ferric ions determined by measuring the optical density with a Beckman DU Spectrophotometer at 304 m μ . This procedure was repeated for three different irradiation times, with five replicates at each time. The total dose for each time was calculated by the method of Spinks and Woods (1964). The dose obtained was plotted against time to give

the calibration curve of Figure 6 which allows the determination of the dose administered for any given irradiation time.

To obtain a dose rate of the correct magnitude for these experiments, the safety door of the irradiator was opened, but the 12 rods of ^{60}Co were not moved up to surround the irradiation chamber. The dose rate for all five tube positions in the tube holder were the same, 150 rads per minute, as shown in Figure 6.

Similar dosimetry determinations were made with the rods raised to surround the irradiation chamber. Figure 7 indicates the results of these determinations, showing a dose rate of 4800 rads per minute with the ^{60}Co rods in this position. This position was used only for the highest dose in the preliminary experiment to determine the correct composition of culture water for these experiments.

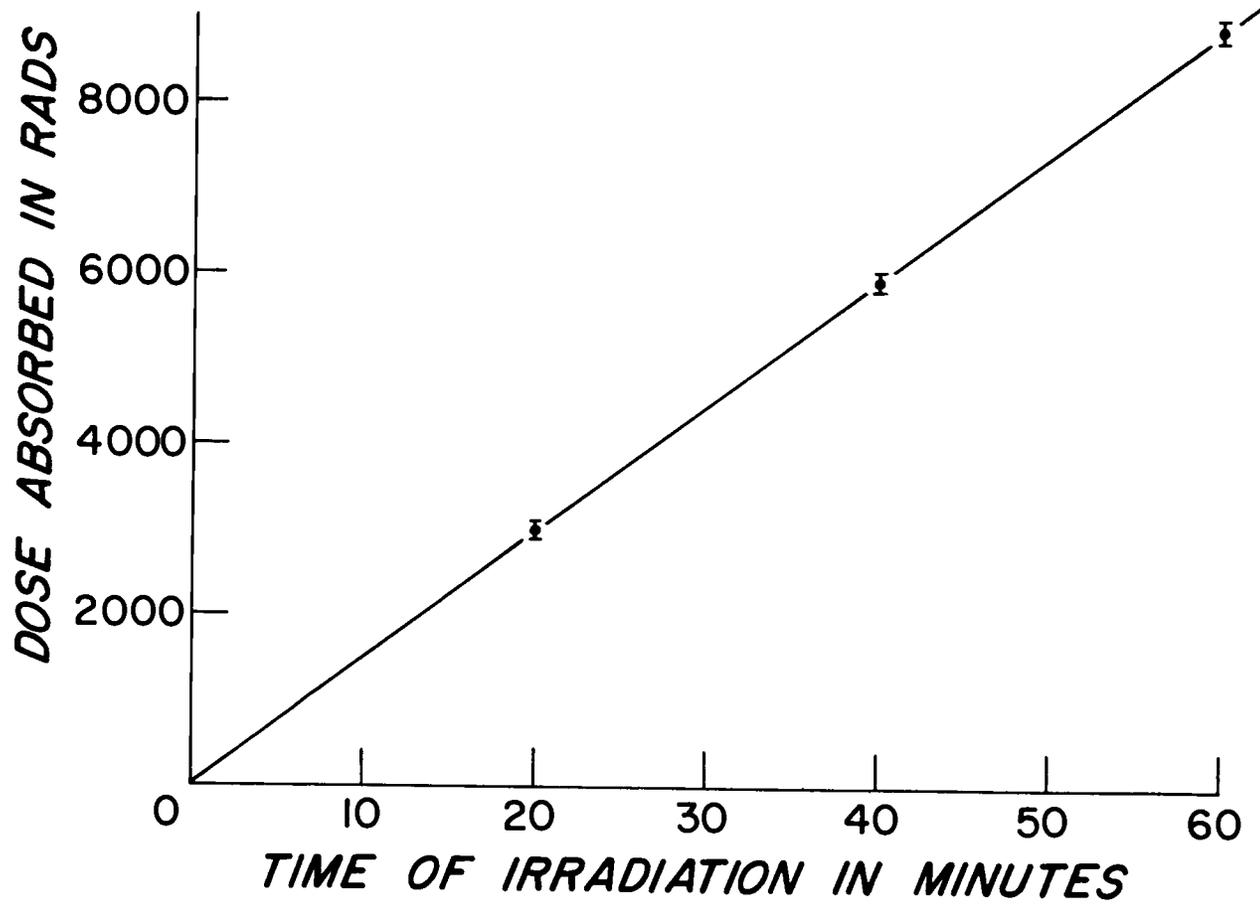


Figure 6. Calibration curve obtained with the ^{60}Co rods in the lowest position.

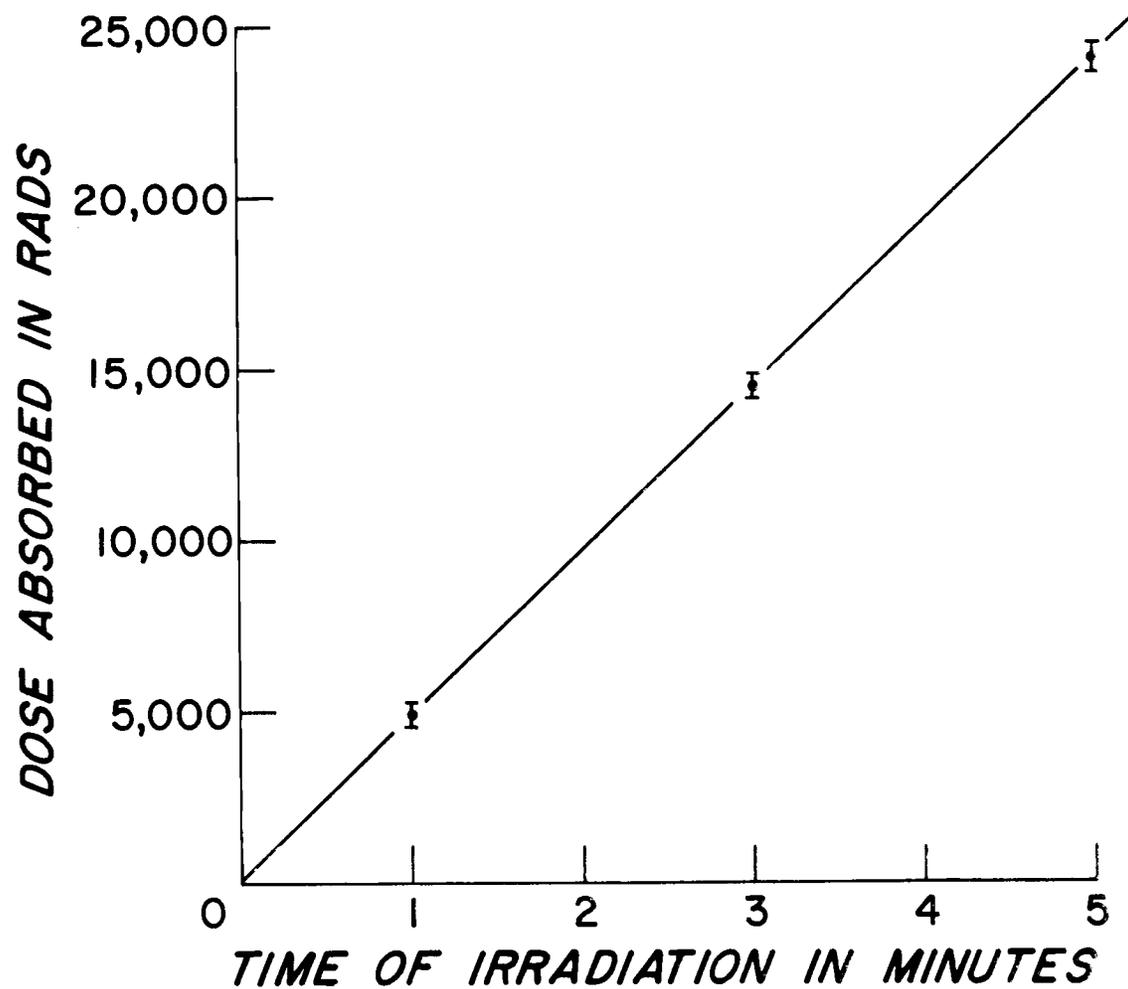


Figure 7. Calibration curve obtained with the ^{60}Co rods in the highest position.

RESULTS AND DISCUSSION

Pair Mating Experiment

This experiment was conducted to study the effects of an acute dose of gamma irradiation, from the ^{60}Co source, upon the reproductive performance of Artemia in individual pair matings. During the course of a series of preliminary experiments, it became very clear that the stage of the life cycle at which the Artemia were irradiated had an important effect on the subsequently observed reproductive pattern.

Irradiation of the early nauplii stages in the life cycle produced marked effects on reproductive ability, of those which survived the irradiation, but only at relatively high doses of approximately 2000 rads and above. However, the irradiation of these nauplii at such doses produced detectable somatic damage. The irradiated nauplii showed a high mortality rate and such doses also slowed their rate of development to adulthood. This lower rate of development effectively eliminated irradiation of nauplii from this study, since animals receiving different doses became reproductively mature at varying times and hence the reproductive performance was confounded with developmental rate in such a way as to make analysis of any data impossible.

As the Artemia approached maturity, their reproductive ability became increasingly more sensitive to the effects of gamma irradiation. At the same time as they were becoming reproductively more sensitive to irradiation, they were showing greatly increased resistance to somatic damage, as evidenced by lowered mortality at the range of doses used in this experiment. This apparent paradox can be relatively easily resolved if one considers what is occurring at the cellular level within the organism during development. During early stages the animal is growing rapidly and developing new tissues by active division of its existing cells. As it approaches the adult state this somatic cell division reaches a minimum level, but at the same time mitotic activity is greatly increased in germinal tissue as the oögonia and spermatogonia proliferate to form new cells which will mature into oöcytes and spermatocytes, undergo meiosis, and form the egg and sperm cells. Accepting the general principle that cells are most sensitive to ionizing irradiations when actively dividing (Bacq and Alexander, 1961), we can readily see why the nauplii are most sensitive to somatic damage and the later stages become more sensitive to damage in the germinal tissues.

However, in the fully matured and reproducing adults, this sensitivity is masked by the release of one or two broods of young by each female which apparently arise from oöcytes and spermatocytes which have already been formed from the oögonial and spermatogonial

cells before irradiation and are awaiting further development within the organism at the time of irradiation. This interpretation is in accord with similar results discussed by Grosch (1965) from work with the wasp, Habrobracon. He concluded that the period of greatest radiosensitivity of the female is during the proliferation of oögonia by mitotic activity. After the oögonia begins the growth and maturation process to form oöcytes, they are much more resistant to irradiation. Habrobracon was especially favorable material for this kind of a study since the ovarioles are arranged in such a way that it is possible to determine rather precisely the stage of development at the time of irradiation.

For this experiment it was considered best to irradiate the animals at a time of maximum sensitivity to gamma rays and yet to irradiate them before the results would be obscured by broods produced from oöcytes and spermatocytes which were formed before the irradiation. A second preliminary experiment resolved the stage of maturity for irradiation necessary to satisfy the above requirements. It was found that irradiation at either the 10th or 11th instar of Heath (1924) would irradiate germinal tissue at a time of maximum radiosensitivity and would still be before any radioresistant germinal products were formed. This also worked out to be the 22nd or 23rd day after the hydration of the eggs under the standard condition employed in this laboratory.

The animals for this experiment were hydrated, hatched and transferred according to the standard technique as previously described when discussing the conditions of culturing Artemia. After hatching they were placed in an eight gallon aquarium tank for development. Twenty-two days after hydration, the animals were removed from the aquarium with the aid of a fish net and a sample was mounted and examined under the microscope to determine the stage of development. It was found that the development was very uniform with a few animals in the 11th instar and between 80 and 90 percent in the 10th instar. The animals were separated into groups of 80 unsexed animals. One group of 80 such animals was irradiated in 10 ml of culture fluid under the standard conditions of irradiation as previously described, at each of the following doses: 0, 300, 600, 900, 1200, 1500, 1800, 2100, 2400, 3000, 4500 and 6000 rads. The smaller preliminary experiments previously mentioned had indicated that the doses at 300 rad intervals to 2400 rads should bracket the entire range of irradiation within which reproduction would be possible. The three higher doses were included in case the animals irradiated at 2400 rads should show any reproductive ability. The control group of 0 rads was placed in the irradiator for the same length of time (40 minutes) as the group receiving 6000 rads, but in this case the safety door was not opened.

After irradiation each sample of 80 animals was placed in a

separate container with one liter of culture water. Mating was accomplished by allowing the Artemia to pair and assume the copulatory posture in the culture water. After the males had clasped the females the pairs were transferred to the individual 500 ml containers and observed daily to record their reproductive performance. This process continued until 20 pairs which had received each of the doses were isolated for further study. The first noted pair clasping occurred four days after irradiation, and by the eighth day after irradiation all of the required 240 pair matings had been isolated.

Mating the animals in this fashion is a form of artificial selection. Those pairs that reach maturity the earliest and hence clasp in mating posture will be selected in preference to those which mature at a later time. Allowing the male to select the female it clasps is a form of natural selection in operation in the laboratory and certainly destroys the concept of random mating, but probably more nearly approaches the mating system that occurs in nature, although we must remember that the late maturing animals are not sampled. A similar mating system has been employed by Grosch (1966). One consequence of this type of mating has been the very high frequency of such matings which produce offspring in the control group and at all doses below 1500 rads. Control animals mated in this fashion have shown that over 95 percent of the pairs produce offspring. This can be compared to 71 percent of the pairs producing

offspring in random control matings reported by Bowen (1964). However, her work is not directly comparable since her mating was in only 5 ml of culture media as compared to the 500 ml used in this experiment.

These 20 pair matings at each of the 12 doses were observed daily during the life span of the female for the production of broods. If a male was noted as dead in a culture, he was replaced by another male which had received the same dose. If a female died, that particular culture was terminated at the time of her death. When a brood was produced, the parents were carefully removed to a fresh culture container and observed daily for the production of subsequent broods.

The number of offspring in each brood was counted on the third day after they were produced. They were again counted and sexed at maturity. Each brood was fed and examined daily. If a particular culture was cloudy due to microbial growth, feeding was withheld until the animals had succeeded in cleaning up the culture. The data obtained in this experiment are summarized in Table 3. This shows that the range of doses employed in the experiment covered the range of reproductive radiosensitivity of Artemia, since with an acute dose of 2100 rads, administered at the time of maximum radiosensitivity the animals are rendered effectively sterile, but survive for essentially as long as the controls.

Table 3. Summary of the effects of various doses of irradiation as determined from pair matings of irradiated animals. Based on 20 pairs at each dose. Means and standard errors are given for all per pair and per brood values.

Dose (rads)	Number of Broods	Broods per Pair	Nauplii Voided			Percent Survival to Adults	Mature Adults		
			Total	Per Pair	Per Brood		Total	Per Pair	Per Brood
0	87	4.35 \pm .31	9,692	484.6 \pm 30.9	111.4 \pm 6.4	64.9	6,290	314.5 \pm 14.5	72.3 \pm 3.9
300	92	4.60 \pm .38	9,916	495.8 \pm 29.4	107.8 \pm 5.3	67.8	6,723	336.2 \pm 13.0	73.1 \pm 3.4
600	89	4.45 \pm .39	10,305	515.2 \pm 38.2	115.8 \pm 6.8	56.4	5,812	290.6 \pm 21.3	65.3 \pm 5.5
900	60	3.00 \pm .30	7,005	350.2 \pm 24.2	116.7 \pm 5.2	59.3	4,154	207.7 \pm 11.1	69.2 \pm 5.1
1200	53	2.65 \pm .38	8,321	416.0 \pm 34.7	157.0 \pm 7.6	50.7	4,219	211.0 \pm 20.2	79.6 \pm 7.2
1500	39	1.95 \pm .28	4,831	241.5 \pm 28.7	123.9 \pm 8.1	40.3	1,947	97.4 \pm 8.3	49.9 \pm 7.2
1800	26	1.30 \pm .27	1,894	94.7 \pm 31.2	72.8 \pm 8.7	31.1	589	29.4 \pm 5.6	22.7 \pm 6.4
2100	9	0.45 \pm .21	237	11.8 \pm 5.7	26.3 \pm 9.2	0	0	0	0
2400	0	0	0	0	0	0	0	0	0
3000	0	0	0	0	0	0	0	0	0
4500	0	0	0	0	0	0	0	0	0
6000	0	0	0	0	0	0	0	0	0

A comparison of this sterility level of 2100 rads for Artemia, with values obtained for insects (Grosch and Erdmann, 1955) would indicate that the reproduction performance of Artemia displays a sensitivity to irradiation which is of the same magnitude as in several orders of insects. Grosch and Sullivan (1955) established a sterility dose by use of x-rays for Artemia females of 2250 rads which is also in agreement with the data presented in this experiment.

The number of adults produced per pair as a function of dosage is plotted for this experiment in Figure 8. The vertical lines included show the magnitude of one standard error of the mean above and below the mean. Several factors apparent from this graph should be discussed.

The relatively higher standard error noted in both the 600 and 1200 rad samples can be largely explained in both cases by the fact that one of the twenty pairs exhibited a very high reproductive capacity, and this single high value caused the standard error of these two dosages to be higher than found in the other cases.

Even discounting these two samples, the standard error of the offspring produced per pair is high. However, there is no reason to believe that this variation noted is an effect of the irradiation since the control group also shows a great variation. Therefore, this variation noted is either due to genetic variability found in this population

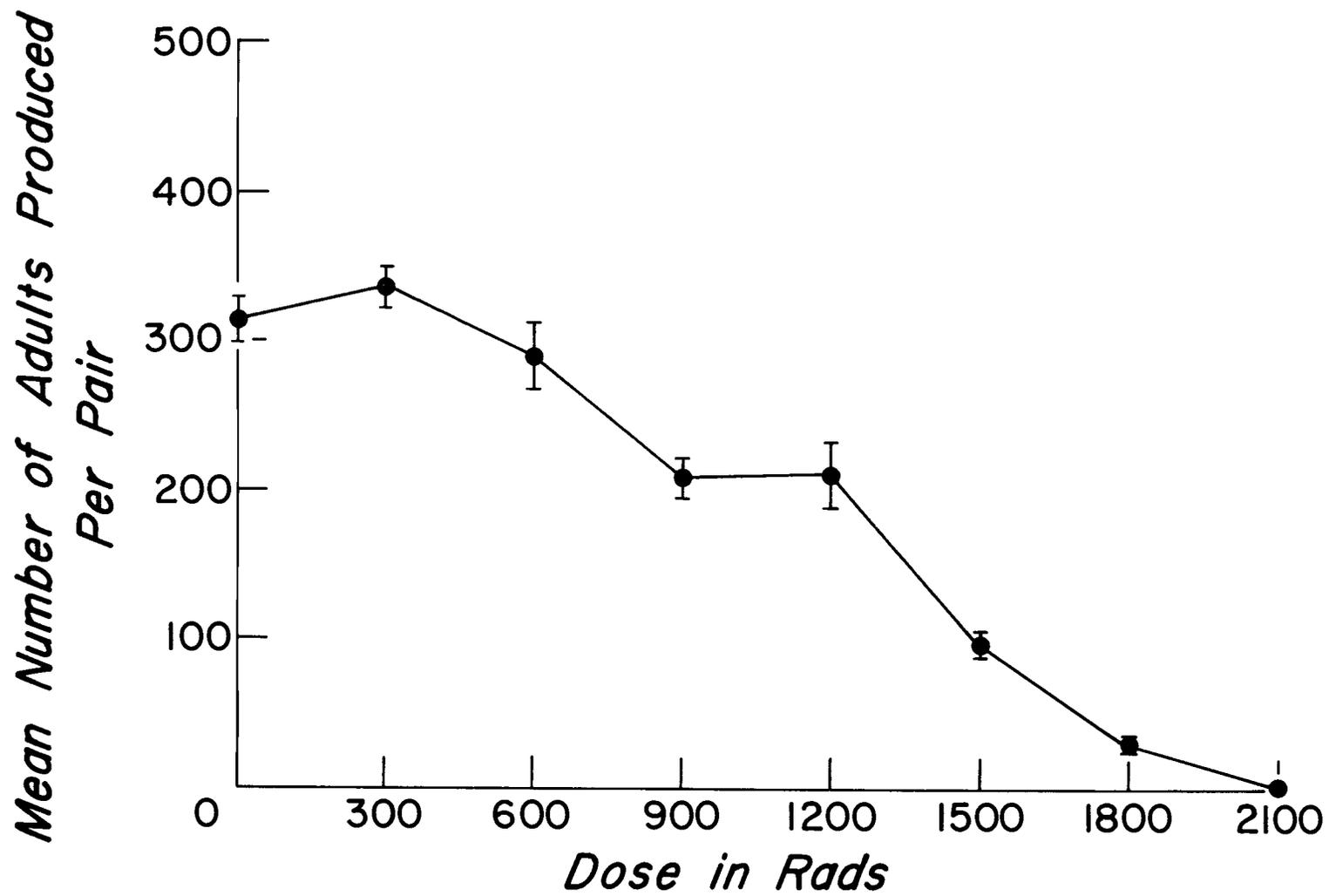


Figure 8. The mean and standard error of the number of adults produced by irradiated pairs of animals.

of shrimp or it may be due to handling of the animals during culturing. The handling has been shown not to shorten the lifespan, but could still be the cause of variability in reproductive performance. At the present time, several lines of shrimp are being inbred through at least ten generations to obtain some highly homozygous lines. If such inbreeding is successful, further experiments can then resolve the question of variability in reproductive performance and assess what role genetic heterozygosity has played in this deviation.

The slight increase in reproduction per pair noted at 300 rads is not statistically significant, but the trend is not wholly unexpected. White et al. (1966) have shown that brine shrimp which received a dose of 500 rads of gamma rays were longer in size during development and matured sexually at an earlier date than control shrimp. Stimulation of growth by ionizing radiation has also been recognized in various other species, such as crabs (Engel, 1967) and amphibians (Brunst, 1965). Such an increased growth and early maturity might also result in increased reproduction as possibly indicated in the current experiments. The reality of such an increase will be examined in the future by the use of inbred strains of Artemia to reduce the within-sample deviations and the resolution can be increased by using a series of several dosages between 0 and 600 rads.

The unexpected lack of decrease in reproduction shown at 1200 rads cannot be rationally explained. The single very prolific pair

which increased the standard error as discussed before, cannot by themselves, account for the change in trend noted at this dosage. It can only be suggested that the true mean value should probably be considerably lower, and in view of the standard error observed, this suggestion does not seem out of line.

The data from Table 3 can also be plotted in terms of the adults produced per brood versus the dosage administered. Such a graph is shown in Figure 9. This shows rather clearly that the number of mature adults produced per brood remains relatively constant until the dosage has reached 1200 rads. Then we see a rapid decrease in the per brood production of adults. Hence, some kind of threshold is present at above 1200 rads. Any dosage below this amount has little effect on the number of adults produced per brood. Viewing the same data another way, we can see from Table 3 that the reduction in net reproductive ability demonstrated at doses of 1200 rads and below as shown in Figure 8, is due primarily to a reduction in the number of broods produced and not to a decrease in the number of adults produced per brood. At 1500 and 1800 rads we see a continued reduction in the number of broods produced as well as a marked decrease in the number of adults per brood.

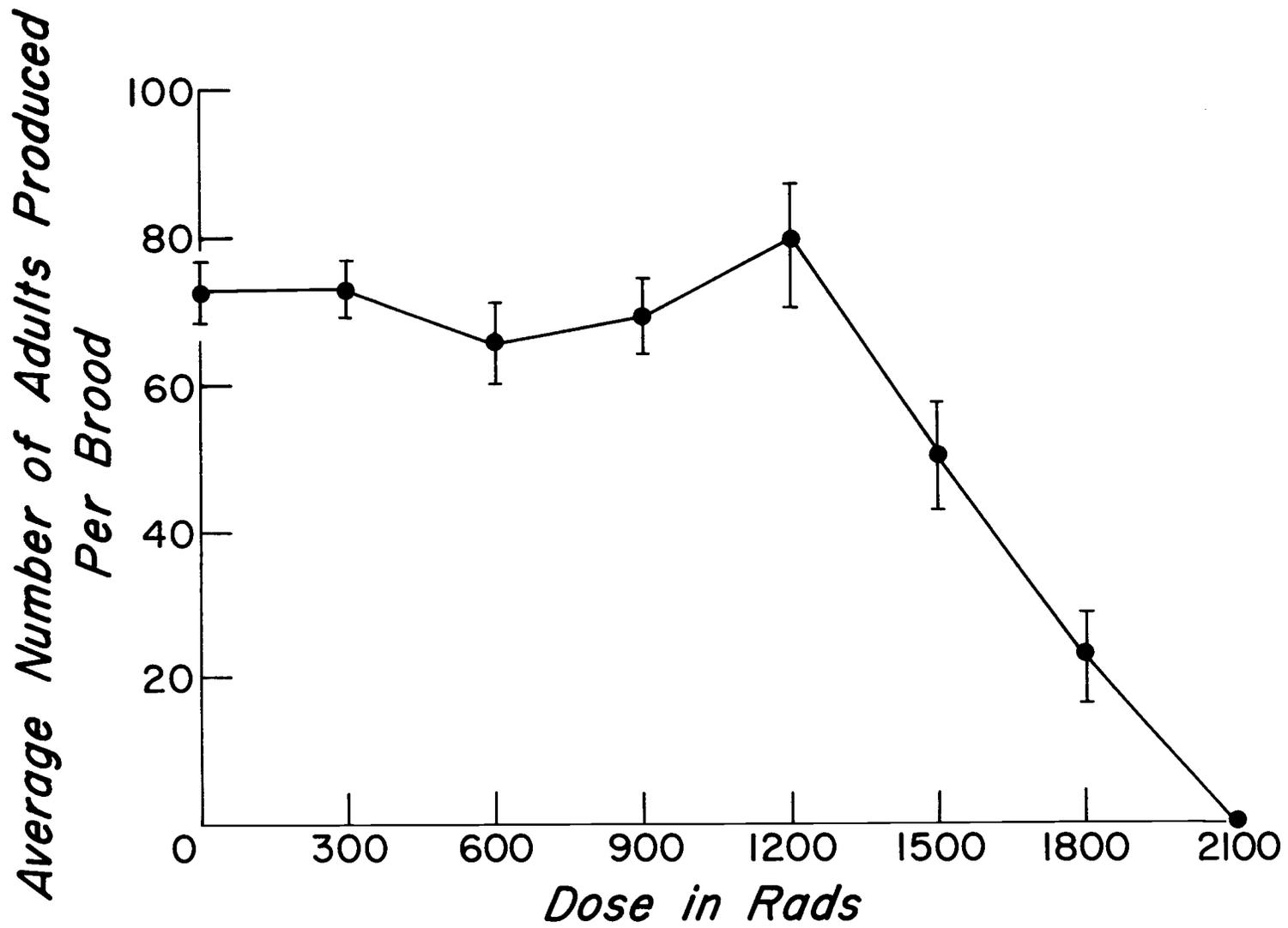


Figure 9. The mean and standard error of the number of adults produced for each brood of nauplii released.

Population Culture Experiment

The pair mating irradiation experiment, described in the preceding section, which required the irradiation of animals at a specific stage of their life cycle allowed more accurate control of experimental conditions than was possible in the experiment to be described in this section. However, such irradiation fails to simulate the conditions of exposure to irradiation that a functioning population might encounter in an ecological setting. In the environment, a typical animal population often consists of all stages in the life cycle being represented at any given time. The population culture irradiation experiment described in this section is an attempt to approach the environmental situation by irradiating a population composed of animals from the 7th instar of Heath (1924) to mature adults which were reproducing at the time of irradiation.

Irradiation of the earlier nauplii stages was not included in this experiment for two reasons. First, at the levels of irradiation to be used, the subsequent reproductive performance of these early nauplii would be confounded with their altered developmental rate, an effect which was previously discussed in regards to the pair mating experiment. A second reason for irradiating only the larger nauplii and adults was because of the impossibility of mechanically separating the smaller stages from the solid waste materials which accumulate

in the population cultures. This waste material is composed primarily of the fecal material excreted by the adults and is the same size as the earlier stages in the life cycle. Because of this problem, it was possible to count only individuals from the 7th instar to the adult state during the later counts of the population culture. Hence, to insure a uniformity in determining the numbers of animals in the cultures, only those stages which could be recovered for later counting were included in the original irradiated sample.

The Artemia irradiated for this experiment were hydrated, hatched and handled by the standard methods. A series of ten different groups of eggs were hydrated every other day, and as they hatched, they were introduced into an eight gallon aquarium for continued development. The aquarium stock was fed daily in amounts necessary to maintain a normal growth rate. When the oldest animals in the aquarium had matured and were starting to produce young, samples of Artemia were prepared for irradiation.

A random sample of animals was removed from the aquarium by use of a dip net, after the animals had been thoroughly mixed to insure a sampling of all sizes of animals. The animals removed were placed on a 1100 μ bolting silk. A double rinse with fresh culture water of the animals on this filter allowed the few early stages present to pass through and retained essentially all animals from the 7th instar to the adults on the bolting silk. The animals retained on the

bolting silk were then divided into groups of 300 animals. This size of culture was chosen in view of the report of Grosch (1962) that as many as 300 well developed Artemia were counted in his three liter population cultures at one time. It was considered advisable to start the cultures as mass populations in this assessment of the effects of irradiation on population maintenance, rather than irradiating a few animals and allowing them to reproduce to form a population at near the carrying capacity of the environment.

Four groups, of 300 animals each, were then selected at random for irradiation by the standard technique at each of the following dosages: 0, 500, 1500 and 3000 rads. The four control groups were placed in the irradiator for the same length of time as the 3000 rad groups, but the safety door was not opened. Each group of 300 irradiated animals was then placed in three liters of standard culture water and placed in a gallon jar to start a separate population culture. All of these population cultures were fed one ml of the standard yeast suspension daily.

The effects of this irradiation upon the population maintenance were studied in two different ways. Three of the cultures at each dosage were examined every four weeks for a 20 week period in the following manner. The entire culture was siphoned out of their container and carefully strained through an 1100 μ bolting silk. The animals on the bolting silk were rinsed twice with water decanted off

the top of the culture to insure removal of all young nauplii. This procedure allowed all of the smaller nauplii to pass through the bolting silk and again retained the animals more mature than the 7th instar. A population count of these stages was then made. After counting was completed, the entire population was returned to the culture container with original culture water for further development. The data obtained from these counts is presented in Table 4.

Table 4. The mean number of 7th instar to adult Artemia found in population cultures receiving various doses of irradiation. Each value is the mean and standard error, based on three replicate population cultures.

Sampling Interval	Dose (rads)			
	0	500	1500	3000
0 weeks	300 \pm 0	300 \pm 0	300 \pm 0	300 \pm 0
4 weeks	578 \pm 29	426 \pm 31	337 \pm 27	284 \pm 27
8 weeks	598 \pm 39	603 \pm 43	341 \pm 15	354 \pm 24
12 weeks	513 \pm 24	627 \pm 20	399 \pm 25	385 \pm 16
16 weeks	453 \pm 25	520 \pm 14	421 \pm 18	344 \pm 25
20 weeks	375 \pm 36	379 \pm 35	362 \pm 20	337 \pm 12

The mean value calculated for each dosage at the four week intervals is plotted in Figure 10. Several things should be noted about this evaluation of the irradiation effects by total population counts. The number of animals found in the control populations had

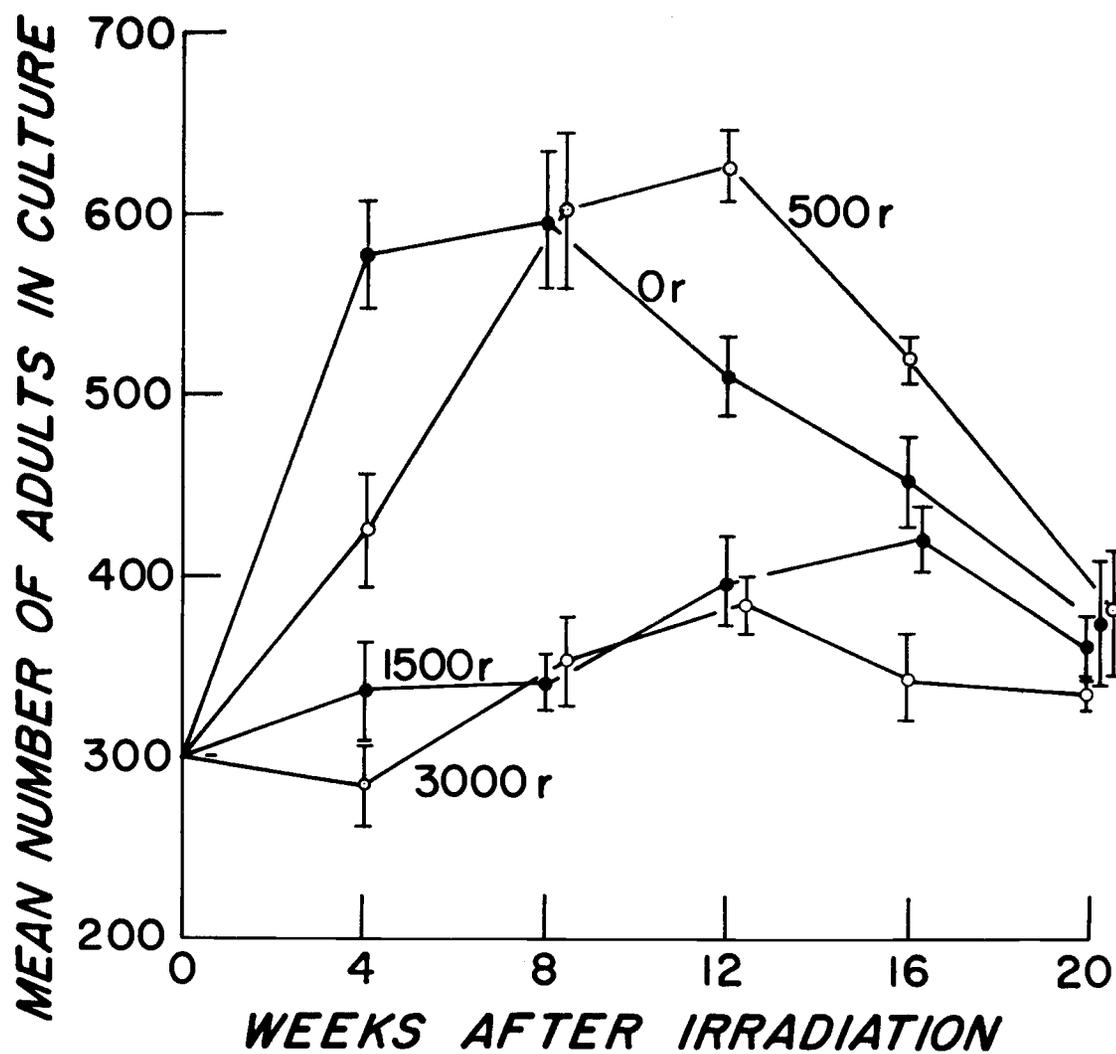


Figure 10. The mean number of seventh instar to adult *Artemia* found in population cultures receiving various doses of irradiation.

virtually doubled at the end of four weeks, indicating a real potential for population growth under these culture conditions. However, a comparison with the control animals in the pair mating experiment shows that only about 1/80 of the reproductive potential inherent in this population of Artemia is realized under these population culture conditions. This fact leads us to conclude that an environmental stress is severely limiting the population size. This conclusion is supported by the observation that the control populations increased only slightly during the next four weeks, and during the final weeks of the experiment the number of animals in the control cultures steadily decreased. Figure 10 shows that this decrease is linear over the final 12 weeks of the experiment and had shown no signs of leveling off at the termination of the experiment. It may be assumed that this control population would have reached an even lower level if the experiment had been continued for a longer period of time.

The three cultures receiving 500 rads showed a pattern of distinct population growth followed by a steady decline which was very similar to the control culture. However, in this case a four week lag in the growth pattern, as compared to the control populations, was observed. In view of the data presented in the pair mating experiment, it is not possible to explain this lag. It was shown that at the most radiosensitive state of the life cycle, a dose of 600 rads

had no significant effect on the number of adults produced per pair, as compared to the control animals, and yet in this experiment we see a marked effect at the end of the fourth week between the control animals and those which received 500 rads. Any explanation for this lagging pattern of population development would need to be evolved in one of the following ways. One explanation might be in terms of an interaction effect between the irradiation and the more crowded conditions of the population culture during the early weeks of this experiment as compared to the uncrowded conditions for growth in the pair mating experiment. An alternative explanation to be studied would of course be to consider the differential effect of irradiating many different stages in the life cycle in this experiment as compared to the more radiosensitive stages in the previous experiment. However, a reference to the discussion of the pair mating experiment concerning this matter would tend to lead to rejection of this hypothesis, since one would expect that the irradiation of all stages in the life cycle, including those more radioresistant stages, would have produced a lesser effect upon the reproductive performance of the population than irradiation of only the most sensitive stages.

The similar decrease in population size noted for both the 0 and 500 rad cultures in the final weeks of the experiment and the fact that the cultures reached almost identical mean values on the 20th week after irradiation may be interpreted to mean that there is some

finite carrying capacity inherent under these culture conditions, and that all the populations are tending to approach this carrying capacity as a limit. Although a population, with little or no damage from irradiation, may temporarily overshoot this carrying capacity, the environmental stress will ultimately mount and reduce the population to a size commensurate with the potentialities of the environment. The precise factor or interaction of factors which are operating to limit the population size under these culture conditions has not been determined, but several possibilities may be considered. These include the lack of an adequate food supply to sustain a larger population, a build up of toxic organic waste products in the culture water or an absolute limitation on the rate at which such products may be broken down and removed from the culture, and finally the population size may be regulated by the rate of gaseous exchange between the culture and the atmosphere. This final factor, rate of gaseous exchange, could certainly act to limit the breakdown and removal of waste products from the culture water and hence, an interaction of these two factors could effectively limit the population size.

The cultures receiving the doses of 1500 and 3000 rads showed the effects of the irradiation during the early weeks of the experiment with decidedly smaller populations than the control cultures, hence for the first 12 to 16 weeks the radiation exposure limited the size of these populations. However, at the end of 20 weeks all of the

populations appear to be approaching a common size, which apparently is controlled by environmental conditions as discussed above.

This might also be used as evidence that since after 20 weeks or about five generations, even the population which received 3000 rads is maintaining itself about as well as the control population, and therefore has essentially recovered from any reproductive damage resulting from the insult with gamma irradiation. However, this hypothesis of little or no reproductive damage being evident after five generations is not supported by the results of the second part of this experiment cited below. It is shown that the individual pairs from the population cultures receiving 1500 and 3000 rads show a significantly lowered reproductive potential when compared to the control cultures. Hence, a more realistic assessment of the situation would be to conclude that after 20 weeks the populations of all four cultures are being limited by environmental factors to essentially the same size. In this case the reproductive damage that is shown to be present by pair matings is masked in the population cultures, and all populations appear to be functioning equally effectively when measured only by a gross population count.

The fourth culture receiving each of the four different doses was handled in a different way. In this case, ten individual pair matings of breeding adults were isolated from each culture at four week intervals. The mated pairs of brine shrimp were randomly

selected from the cultures. As a pair appeared at the top of the culture, it was removed with a small dip net and placed in a 500 ml container where their reproductive performance was studied for the life span of the female, with the same technique as was used in the pair mating experiment. Such a method of selecting pairs of Artemia for study chooses only those pairs at the surface of the culture, and if such pairs are not a random sample of all pairs in the culture, this procedure is biased against those pairs which spend a majority of their time at the bottom of the culture. This procedure would be especially biased if the pairs swam at the surface of the culture only at a certain age and therefore eliminated other age classes from the sample. However, inspection of the pairs obtained in this way showed that various age classes were represented in the samples.

The data obtained from the study of the pair matings made from the population cultures is summarized in Table 5. However, it is much more informative to extract specific values from Table 5 and to plot these values to study various individual components of the reproductive performance of Artemia and, therefore, to assess the relative importance of the various parameters in the reproductive performance of the species.

Figure 11 shows the mean number of broods produced by each pair removed from the cultures for study during the course of the experiment. Since it was shown in the pair mating experiment that

Table 5. Summary of the effects of various doses of irradiation on population cultures of Artemia, as determined by pair matings from the population cultures. Based on ten pairs at each sampling interval. Means and standard errors are given for all per pair and per brood values.

	Number of Broods	Broods per Pair	Nauplii Voided			Percent Survival to Adults	Mature Adults		
			Total	Per Pair	Per Brood		Total	Per Pair	Per Brood
<u>3000 rads</u>									
0 weeks	26	2.6 \pm .44	1,821	182.1 \pm 31.2	70.0 \pm 11.8	20.9	381	38.1 \pm 20.4	14.7 \pm 12.4
4 weeks	6	0.6 \pm .37	671	67.1 \pm 28.0	111.8 \pm 16.8	22.9	154	15.4 \pm 12.0	25.6 \pm 14.7
8 weeks	7	0.7 \pm .38	664	66.4 \pm 27.9	94.9 \pm 15.2	26.7	177	17.7 \pm 12.3	25.3 \pm 18.3
12 weeks	11	1.1 \pm .40	837	83.7 \pm 28.5	76.1 \pm 10.1	27.4	229	22.9 \pm 20.6	20.8 \pm 14.5
16 weeks	9	0.9 \pm .41	796	79.6 \pm 27.0	88.4 \pm 11.0	33.5	267	26.7 \pm 19.8	29.7 \pm 16.8
20 weeks	16	1.6 \pm .53	1,078	107.8 \pm 28.8	67.4 \pm 12.3	37.7	406	40.6 \pm 21.1	25.4 \pm 13.5
<u>1500 rads</u>									
0 weeks	29	2.9 \pm .52	2,983	298.3 \pm 35.6	102.9 \pm 11.8	41.8	1,247	124.7 \pm 30.5	43.0 \pm 11.1
4 weeks	16	1.6 \pm .45	1,516	151.6 \pm 29.4	94.8 \pm 13.0	33.3	505	50.5 \pm 20.7	31.6 \pm 6.8
8 weeks	20	2.0 \pm .68	1,873	187.3 \pm 30.9	93.6 \pm 11.4	34.7	650	65.0 \pm 17.7	32.5 \pm 8.9
12 weeks	23	2.3 \pm .47	2,008	200.8 \pm 31.0	87.3 \pm 12.7	44.6	896	89.6 \pm 16.0	39.0 \pm 7.4
16 weeks	24	2.4 \pm .52	2,367	236.7 \pm 30.3	98.6 \pm 12.5	41.8	989	98.9 \pm 14.1	41.2 \pm 9.0
20 weeks	29	2.9 \pm .40	2,739	273.9 \pm 33.5	94.5 \pm 12.3	51.0	1,397	139.7 \pm 18.9	48.2 \pm 7.5

(continued on page 54)

Table 5 (continued)

	Number of Broods	Broods per Pair	Nauplii Voided			Percent Survival to Adults	Mature Adults		
			Total	Per Pair	Per Brood		Total	Per Pair	Per Brood
<u>500 rads</u>									
0 weeks	40	4.0 \pm .63	4,444	444.4 \pm 39.7	111.1 \pm 11.7	55.6	2,471	247.1 \pm 24.5	61.8 \pm 6.7
4 weeks	27	2.7 \pm .53	3,789	378.9 \pm 38.2	140.3 \pm 11.9	53.2	2,016	201.6 \pm 20.2	74.7 \pm 6.1
8 weeks	33	3.3 \pm .66	3,435	343.5 \pm 36.7	104.1 \pm 9.3	54.4	1,869	186.9 \pm 23.8	56.6 \pm 8.1
12 weeks	31	3.1 \pm .37	3,678	367.8 \pm 28.1	118.6 \pm 10.4	56.6	2,082	208.2 \pm 22.2	67.2 \pm 7.3
16 weeks	36	3.6 \pm .48	3,798	379.8 \pm 37.8	105.5 \pm 9.8	54.7	2,078	207.8 \pm 27.1	57.7 \pm 6.0
20 weeks	39	3.9 \pm .70	4,259	425.9 \pm 42.9	109.2 \pm 10.5	59.0	2,513	251.3 \pm 21.9	64.4 \pm 5.7
<u>0 rads</u>									
0 weeks	42	4.2 \pm .64	4,636	463.6 \pm 42.6	110.4 \pm 13.0	62.1	2,879	287.9 \pm 27.3	68.5 \pm 7.8
4 weeks	39	3.9 \pm .56	4,121	412.1 \pm 37.8	105.7 \pm 12.7	70.1	2,889	288.9 \pm 24.0	74.1 \pm 6.7
8 weeks	38	3.8 \pm .41	4,227	422.7 \pm 38.9	111.2 \pm 12.4	65.6	2,773	277.3 \pm 23.5	73.0 \pm 7.2
12 weeks	43	4.3 \pm .65	4,239	423.9 \pm 41.1	98.6 \pm 11.0	60.1	2,548	254.8 \pm 26.4	59.3 \pm 7.1
16 weeks	38	3.8 \pm .61	3,712	371.2 \pm 39.3	97.7 \pm 11.2	67.4	2,509	250.9 \pm 21.6	66.0 \pm 6.8
20 weeks	42	4.2 \pm .55	4,133	413.3 \pm 40.1	98.4 \pm 10.9	62.0	2,562	256.2 \pm 24.1	61.0 \pm 8.5

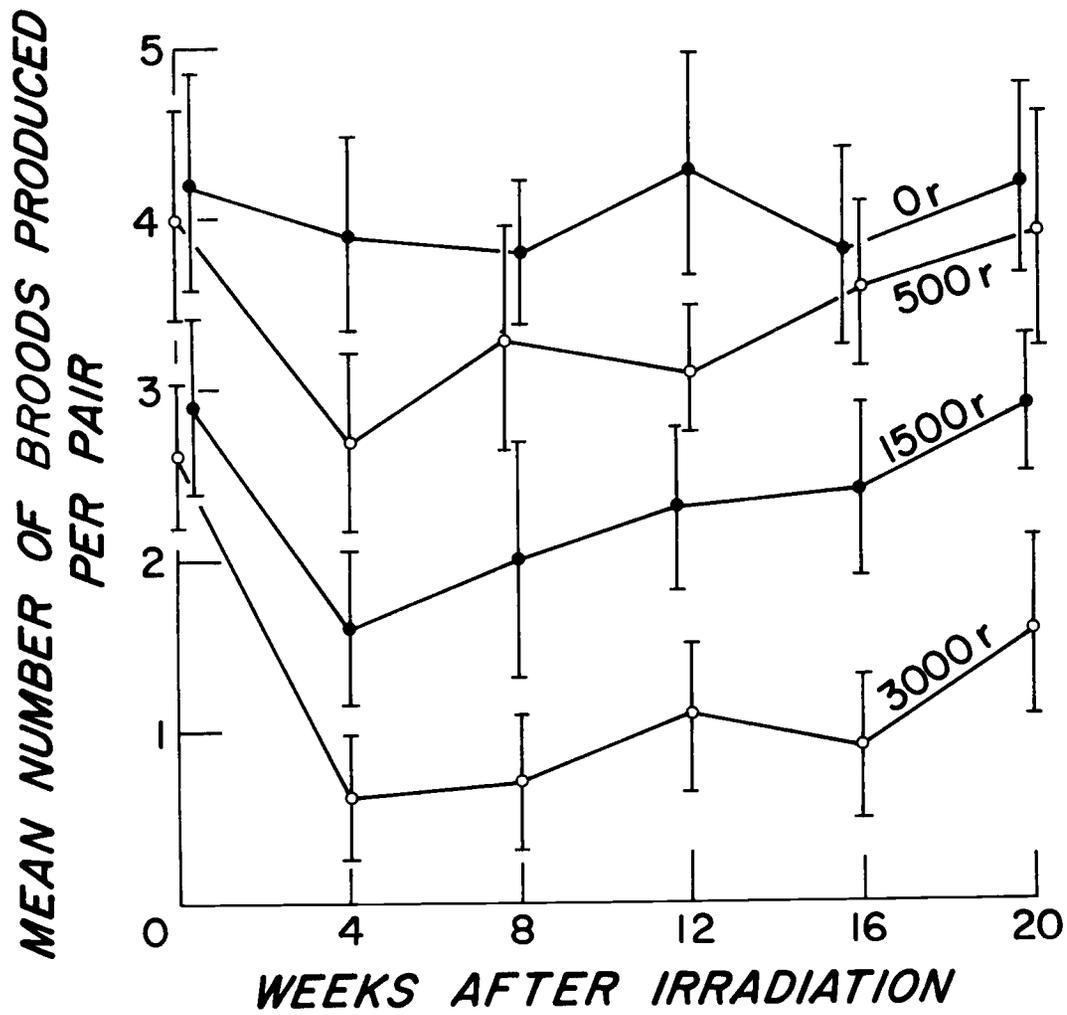


Figure 11. The mean and standard error of the number of broods produced per pair of *Artemia*, removed from irradiated populations at various time intervals.

brood production was the most sensitive parameter when Artemia were insulted by irradiation, it is interesting to note that in this study the brood production is also shown to be greatly affected by the doses of gamma rays involved. Several specific facts can be seen in Figure 11. The brood production was affected relatively little at the first sampling period, which occurred within six hours of the time the animals were irradiated. At this time many females already had one or two broods of eggs which were maturing in their oviducts or uteri. The fertilization and release of these eggs provided the relatively less affected brood production at this first sampling interval.

At the four week sampling period we see a significant decrease in brood production for all irradiated samples. The decrease for the 500 rad group stands in contrast to the 600 rad group in the pair mating experiment, where no significant decrease in brood production was noted. The only explanation that can be advanced to explain this phenomenon would be that the effects on brood production will tend to show up to a greater degree within the first filial generation that was sampled at four weeks in this experiment than in the generation which was exposed to the irradiation. The importance of this possibility would suggest that it warrants intensive study in the future.

The principal interest in this population culture irradiation experiment was to ascertain the degree of recovery of reproductive

performance at successive intervals after irradiation. Figure 11 shows us that the brood production after the fourth week began a slow but constant recovery. However, even after 20 weeks, or about five generations, we see that the culture which received 3000 rads was still producing less than half as many broods as the control culture, and the culture which received 1500 rads was only producing 75 percent as many broods as the control culture. Therefore, we can conclude that at doses of 1500 rads or over that more than 20 weeks, or five generations, are required for the brood production to return to the control level.

At 500 rads we see the same pattern of recovery. However, in this case the population had recovered by the 16th week, with the brood production at the 16th and 20th weeks not significantly different than the brood production for the control groups.

A second parameter involved in the reproductive performance is plotted in Figure 12. Here we see that the number of nauplii produced on successive weeks by various samples of females from irradiated populations shows essentially the same trend as seen in brood production. However, we do note some differences. The animals from the 3000 rad culture show no significant increase in nauplii production from the 4th to the 20th week, even though the number of broods produced was shown to increase. Hence, our attention is called to Table 5, where we confirm this trend by noting a decrease

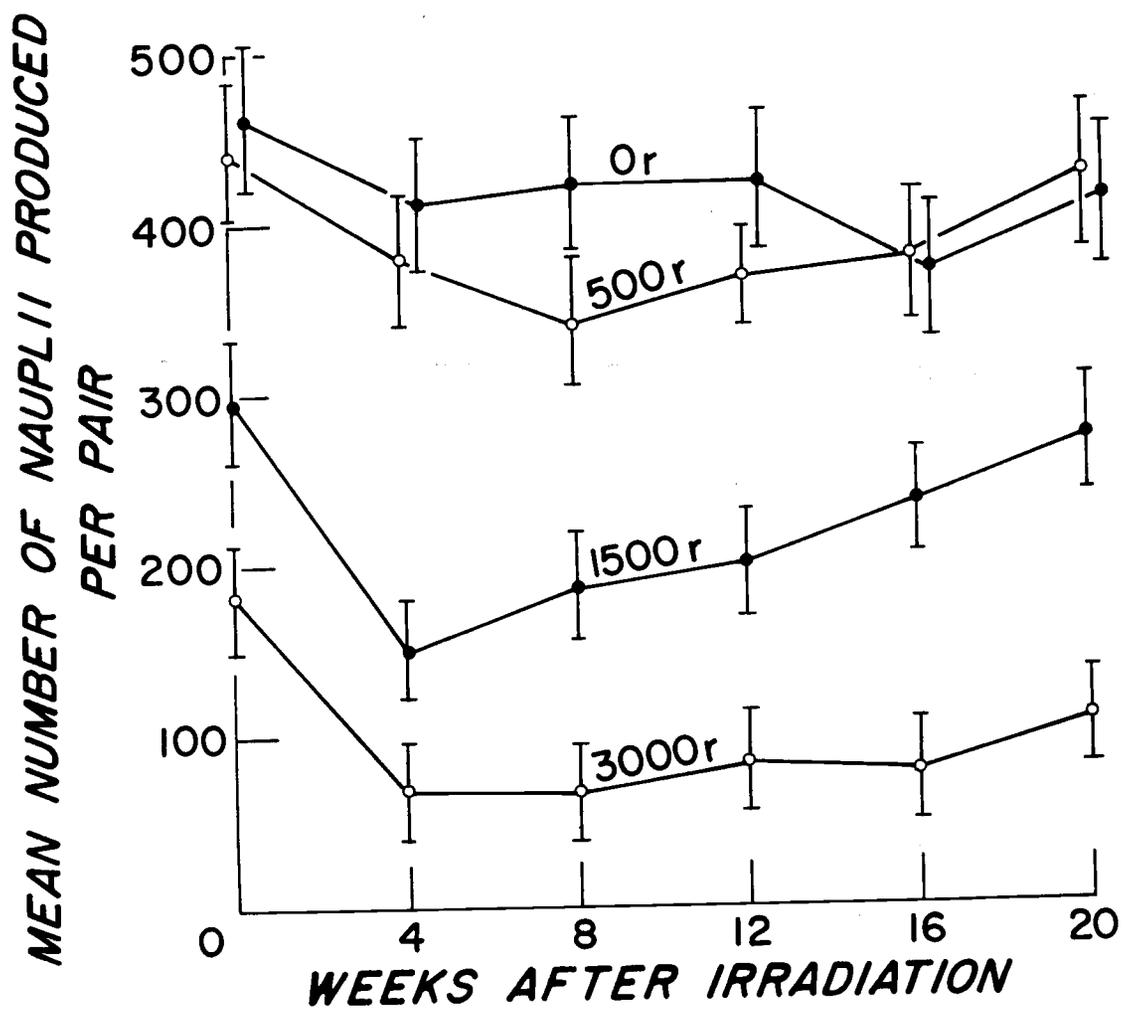


Figure 12. The mean and standard error of the number of nauplii produced per pair of *Artemia*, removed from irradiated populations at various time intervals.

in the number of nauplii produced per brood from the 4th to the 20th week. No explanation is apparent to explain such a trend. The difference in number of nauplii produced between the control and the 500 rad culture is shown to be slight at all times. However, the 1500 rad culture shows a significant decrease in nauplii production and also shows again that after 20 weeks the population has not recovered from the reproductive damage due to irradiation.

A third factor contributing to total reproductive performance is revealed by Figure 13. Here the percentage of nauplii produced which survive to adulthood are plotted. The control population is shown to rather consistently have a 65 percent survival of nauplii. This value is higher than average control survival reported by Grosch in 1962, but would be well within the range of survival values that Grosch (1966) reported in later experimental work.

The percent surviving for each of the experimental groups shows a general tendency to increase at successive sampling periods with the population irradiated at 3000 rads, showing a steady increase in survival. At 1500 rads, we see the same trend of improved survival from the 4th week to the 20th week. However, it is interesting to note the difference between the 3000 and the 1500 rad populations at the zero and four week sampling intervals. The 1500 rad population shows a very definite decrease in survival between these two periods. However, with the 3000 rad sample we see that the lowest

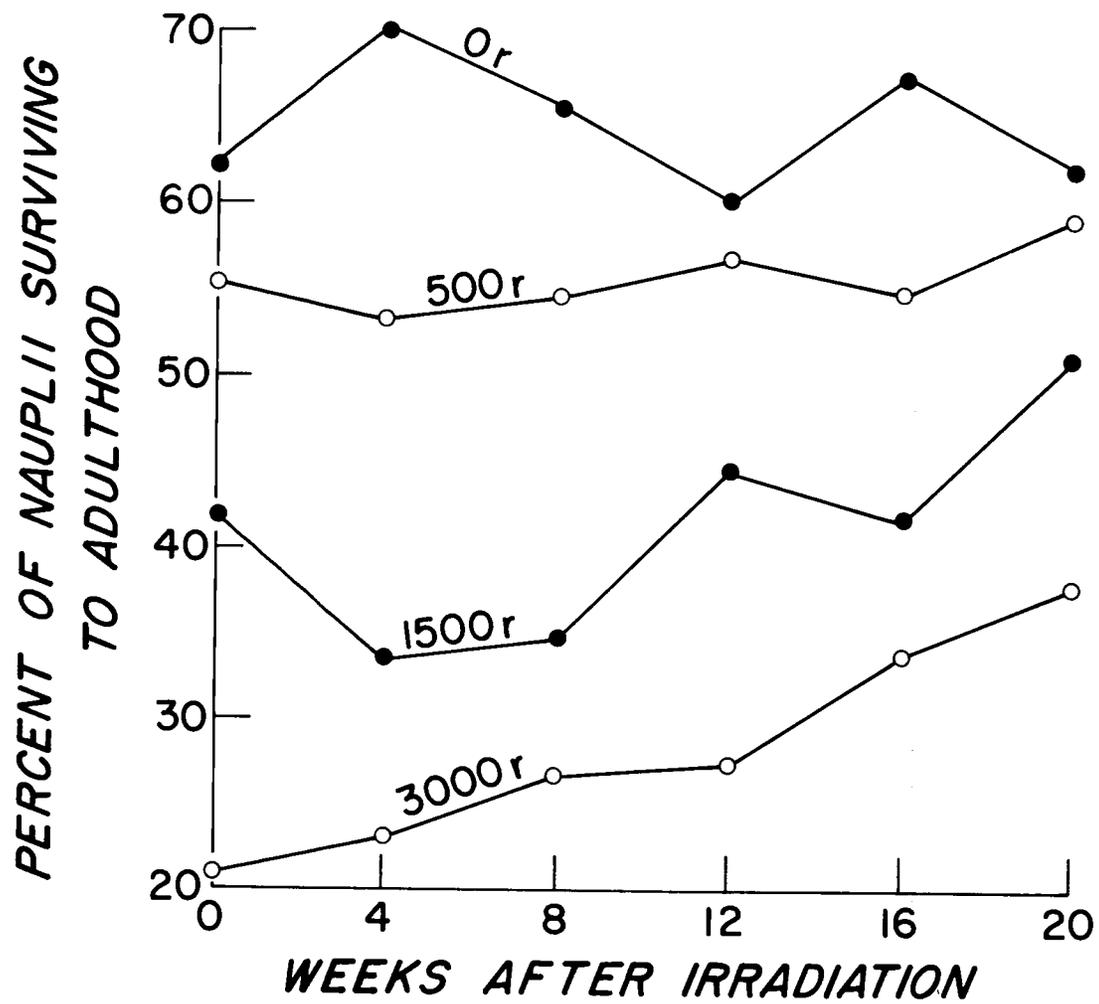


Figure 13. The percent of nauplii, produced by parents removed from irradiated populations at various time intervals, surviving to adulthood.

survival rate occurred at the first sampling period and that at four weeks a slight improvement was already noted. This differential pattern of survival at the two doses may be interpreted to indicate that different mechanisms are operating in determining the survival rate at the different times. At the first sampling period, survival is a function, at least in part, of the ability of zygotes already formed to withstand the effects of irradiation. Figure 13 shows that at this sampling period, 3000 rads is responsible for a pronounced decrease in survival of such zygotes when compared to the survival at 1500 rads. However, at the end of four weeks the survival rate should be interpreted as a measure of the amount of genetic damage carried in the germinal tissue, and we see that at this time the difference between the effects on survival of 3000 and 1500 rads is much smaller.

The population which received 500 rads also shows a slight decrease in survival rate in Figure 13. More interesting is the fact that at this dosage the rate of recovery is very slow indeed. This may suggest that a slightly depressed survival rate for nauplii may occur at doses as low as 500 rads, and complete recovery to the control rate of survival can be attained only after several generations have occurred.

Another parameter is presented in Figure 14. Here, the number of mature adults produced per brood is the same for both the

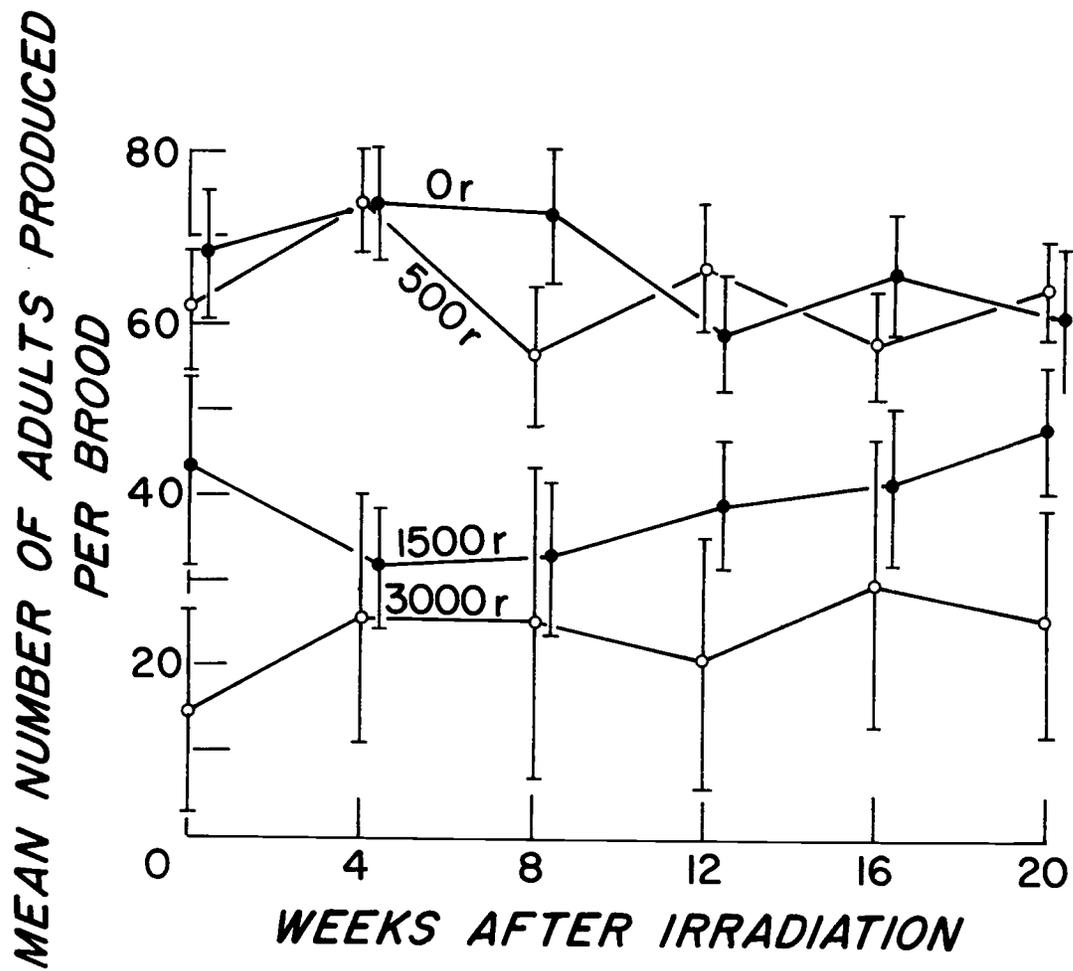


Figure 14. The mean and standard error of the number of adults produced per brood by *Artemia*, removed from irradiated populations at various time intervals.

control and the 500 rad populations. Only at the 1500 and 3000 rad doses do we find the number of adults produced per brood decreased due to the effects of the irradiation. Of special interest is the pattern exhibited by the 1500 and 3000 rad populations at zero and four weeks. We see the same pattern, as noted in Figure 13, when considering survival rates for nauplii, again reflecting itself in the number of adults produced per brood.

It should also be noted that at four and eight weeks the difference in number of adults produced per brood is very slight for the 1500 and 3000 rad populations. However, from the 12th week to the end of the experiment, we note that the 1500 rad culture makes a marked improvement in the number of adults produced per brood, while the 3000 rad population shows very little improvement. Apparently the 3000 rad population has suffered a form of damage which will require many generations before the number of adults produced per brood will return to the control levels.

The final parameter is really the factor of ultimate concern and depends upon each of the previously discussed components of the reproductive ability. This net reproductive potential within the laboratory conditions of this experiment is presented in Figure 15 as the total number of sexually mature adults which are produced by each pair of Artemia from the irradiated populations.

We note that the pair matings isolated from the control culture,

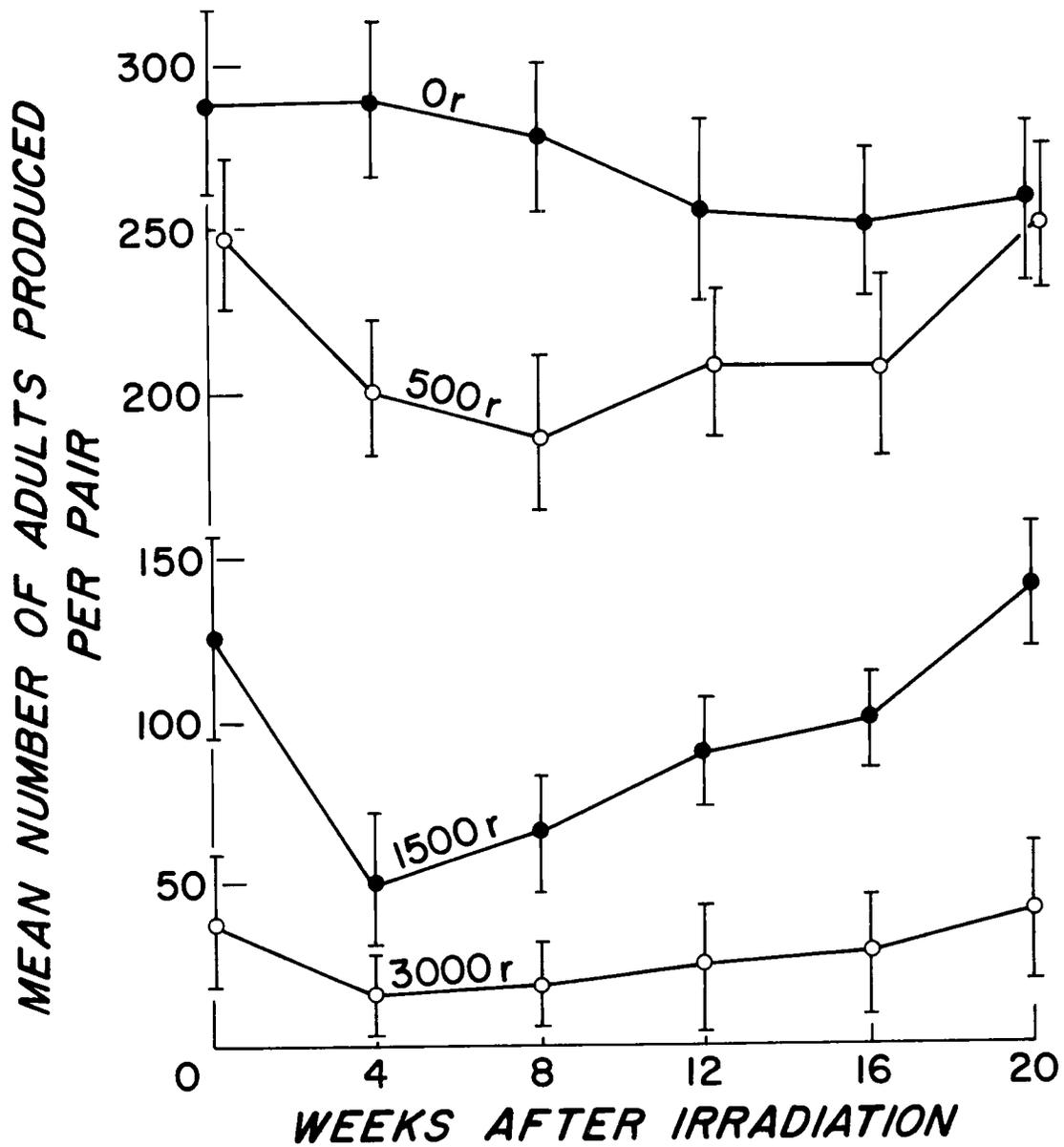


Figure 15. The mean and standard error of the number of adults produced per pair of Artemia, removed from irradiated populations at various time intervals.

on the average, produce about 270 sexually mature adults per pair. The lower production exhibited during the final three sampling periods as compared to the first three sampling intervals cannot be readily explained. The population culture exposed to 500 rads suffered an initial significant depression in net reproductive rate, but the results indicate that it has approached complete recovery at the end of 20 weeks.

In the case of the culture which was exposed to 1500 rads, we see that the net reproductive rate dropped to less than 20 percent of the control rate four weeks after irradiation. It then began a progressive recovery and, by the final week of the experiment, was exhibiting about 50 percent of the reproductive potential of the control culture.

The production of mature adults by pair matings from the culture receiving 3000 rads dropped to less than 10 percent of the control culture. Although this culture did exhibit an improvement in reproductive performance over the course of the experiment, at the final sampling the reproduction was still only about 20 percent of the rate exhibited by the controls. It is important to point out that the rate of recovery of net reproductive ability is much greater for the 1500 rad population than it is for the 3000 rad population.

The results presented in Figure 15 show that a real decrease in reproductive potential is experienced in both the 1500 and 3000 rad

cultures and is still present at the end of 20 weeks. These results stand in contrast to the results obtained by counting the total populations of similar cultures as shown previously in Figure 10 (page 47). The total population counts indicated the 1500 and 3000 rad cultures were maintaining their population size as well as the control culture by the end of 20 weeks. However, we see here that though they were maintaining a normal population size, they still show the effects of irradiation by the display of this lowered reproductive potential.

CONCLUSIONS

The standard conditions of culture employed during the course of these experiments produced a very uniform environment for the Artemia cultures. This very uniformity, which allows us to assess the effects of radiation in the laboratory, makes it difficult indeed to extrapolate these findings to a field population. Such a population would be subjected to variations in temperature, salinity, food supply and many other conditions which could affect the response of the population to radiation. For example, the data previously presented in Table 2 imply an interaction effect between irradiation and the salinity of the culture water when considering the life span of Artemia. Such an interaction effect occurring in the environment might alter conclusions based on controlled laboratory studies. Therefore, we must exercise a certain degree of caution in applying the results of this laboratory study to a population in the field.

A consideration of the results of both the pair mating and population culture experiments clearly demonstrate that the dosage required to stop reproduction depends on the stage of the life cycle being irradiated. If the most sensitive stage, young adults, is irradiated about 2000 rads appears to be the critical level. However, in irradiating a culture of all stages from the 7th instar to adults, we can see that more than 3000 rads is required to produce total sterility.

The observed effect of a particular dose of irradiation depends on the nature of the observation. Counts made of population cultures which received different doses may show the same population levels and hence indicate that those populations which have received the higher doses have recovered from the effects of irradiation. Pair matings from these same cultures have been shown to indicate very different reproductive abilities between the populations which have received different doses. This fact should be kept in mind, especially when thinking about the effects of irradiation in the environmental situation. Even if population cultures, under ideal laboratory conditions, show no effects of the irradiation at a particular dose, it is possible that their reproductive potential has been decreased. This same dosage might affect the functioning field population which is subjected to competition with other species as well as environmental stress. Under these environmental conditions, a population could conceivably be driven to extinction with a radiation dose that showed little or no effect in a laboratory population culture.

The results presented by the analysis of pair matings in the population culture experiment indicated that after a relatively long time of 20 weeks or about five generations, the populations which received doses of 1500 and 3000 rads were still far from recovering completely from the effects of irradiation. This is in agreement with the results of Grosch (1962, 1966) who concluded that a minimum of

12 generations must elapse before a population culture which has survived the addition of $30 \mu\text{Ci } ^{32}\text{P}$ per three liter culture can recover to the point of surviving the addition of a second dose of $30 \mu\text{Ci } ^{32}\text{P}$.

The differences noted between the response to irradiation exhibited by pair matings as compared to the response of population cultures, leads us to conclude that some interaction between the crowded conditions of population cultures and the effect of irradiation on the reproductive performance of Artemia must occur. In the pair mating experiment, a dose of 600 rads was shown to have little effect on the reproductive ability of Artemia. However, in the population culture experiment, a dose of 500 rads was shown to produce a lower population at the end of four weeks than the control population, although this population was able to equal the control population at later sampling intervals.

Further study is suggested in certain areas by the results presented here. The results discussed earlier, indicating that the effects of irradiation on the number of broods produced may be greater with the first filial generation than with the generation that was exposed to irradiation, are important enough to warrant intensive study in the future. The levels for the production of sterility and for effecting various parameters of total reproductive performance can be determined more precisely by using a series of more

closely spaced doses to refine the resolution of the present experiments. It would also seem necessary to extend the present experiment by the use of a low level source to provide for chronic irradiation of the Artemia population. Such experiments would allow interesting comparisons, both with this research and with the results of Grosch (1966).

In conclusion, we should note that the results presented here would tend to confirm the theory that irradiation at the levels presently occurring in the environment will have little or no detrimental effect on the reproductive ability of animal populations. However, the results do indicate that in the event of greatly increased environmental contamination, that any reproductive damage sustained will persist over a long period of time before the populations can recover their full reproductive potential. During the course of such recovery, the nature of the existing ecosystem might be irreversibly altered.

Finally, the very interesting suggestion of a slightly increased reproductive performance at 300 rads, in the pair mating experiment, should be carefully evaluated in further detailed studies. This indication of increased reproduction, following the recently reported enhancement of growth due to irradiation (Brunst, 1965; Engel, 1967; White et al., 1967) should indicate the need for a very careful study of the effects of low level irradiation. If such an enhancement of

reproductive ability could be substantiated in further work, it would require a reassessment of our concepts of the interaction of irradiation with living tissue.

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