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THE EFFECTS OF CONTINUOUS GAMMA RADIATION
ON THE INTRINSIC RATE OF NATURAL INCREASE
OF DAPHNIA PULEX

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INTRODUCTION

This is a study of the effects of continuous, sub-lethal gamma radiation on a particularly significant parameter of living populations, namely, the intrinsic rate of natural increase, customarily symbolized by \underline{r} . The intrinsic rate of natural increase of a population is its potential relative rate of increase in an environment which remains constant on an individual basis. An algebraic definition of the intrinsic rate of natural increase, in terms of any given life-history pattern of age-specific survival and fertility rates, is given by equation (2) in Appendix A. The approach employed here makes it possible also to analyze the effects of the radiation on population attributes underlying \underline{r} . These include the birth rate, \underline{b} , the death rate, \underline{d} , and the stable age-distribution. The effects of radiation on these and other attributes pertaining to the population level of biological integration must be understood in order to predict more accurately the ecological consequences of increased levels of radiation in the biosphere. Although emphasis is placed on the ecological interpretation of the effects of radiation on these population attributes, the relations of these effects to the effects on individual attributes will also be discussed. The latter include age-specific rates of survival and fertility, growth rates, and average life-span.

The results for population attributes are calculated from experimental life table data and refer to theoretical populations since cohorts of a single generation were cultured rather than actual, exponentially-increasing populations. Nevertheless, both the cultures and the theoretical populations associated with them will be referred to simply as "populations."

It was mainly in view of the predictive value and special significance of the intrinsic rate of natural increase in population and community ecology (and in population genetics) that the present approach was taken in a study of the effects of radiation. The period devoted to the experimental work in this study extends from November, 1958 through March, 1960.

The history of ecological concepts involving the intrinsic rate of natural increase perhaps can be said to begin with the famous essay on population by Thomas Malthus (1798). He emphasized the fact that a population, if unchecked, increases in a geometrical ratio. On the other hand, he declared that the food supply could not continue to support such an increase, and the growth of the population would become checked by severe competition for food. The writings of Malthus are recognized as an important contribution to the development of Darwin's theory of evolution. The inherent power of geometrical increase in a population and its ultimate self-limitation were believed by Darwin (1859) to lead to a "struggle for existence" and "survival of the fittest."

Little more was accomplished in the way of testing or improving the theory of the "struggle for existence" for the next fifty years. The first step towards increasing rigor in the ecological theory of competition was Volterra's (1927) application of the logistic equation in a mathematical model of competition between two species competing for a common food supply. This model was later rearranged using different constants and submitted to extensive experimental testing by Gause (1934). The logistic equation had been developed

earlier as a suitable description of human population growth (Verhulst, 1839; Pearl and Reed, 1920), but ecologists, particularly Chapman (1931), did not immediately recognize the application of this equation to the concepts of "biotic potential" and "environmental resistance."

In the competition model of Gause (1934) the intrinsic rates of natural increase do not by themselves predict the outcome of competition. The way in which the rates of increase are affected by a change in population density is also involved. This is better seen in the original form of the competition equations of Volterra. In these, the outcome of competition is predicted simply from the intrinsic rates of natural increase and the effect of density on these rates. Furthermore, as pointed out by Slobodkin (1958), pure competition is probably rare in nature; additional controlling factors are usually present, such as disease or predators. In the case of a nonselective predator feeding on a mixture of prey, the higher the predation rate, the less competitive the situation becomes, and the intrinsic rates of natural increase become more important in determining which of the prey will be eliminated.

The intrinsic rate of natural increase is also important in the theory of predator-prey or parasite-host relationships. Identical mathematical models for these relationships were developed independently by Lotka (1920) and Volterra (1927). The application of this model in experimental studies has been less successful than the competition model (Gause, 1934). However, if enough is known of the influence of environmental factors on the intrinsic rates of natural increase, predation rates, and death rate of predators, this model does have predictive value in special cases. An approach along these lines has been applied

to prediction of phytoplankton-zooplankton relationships in the sea (Riley and Bumpus, 1946; Riley, 1953).

In addition to holding an important place in mathematical models of population dynamics, the intrinsic rate of natural increase has become associated with several other concepts. One of these is Smith's (1954) concept that a species' maximum intrinsic rate of natural increase is a carefully molded product of evolution and a capacity that must exist if the species is to survive. The logic of this stems from the common observation that in most species the average actual rate of increase, taken over a few years, is essentially zero. Therefore, the average "harshness" of the environment of a species may be measured by its maximum value of \underline{r} . Smith also makes note of the correlation between a species' \underline{r} and its size and phylogenetic position. Small, primitive organisms have high values of \underline{r} , while large and more advanced species tend to have proportionately lower values of \underline{r} .

In his Genetical theory of natural selection, Fisher (1930) adds further meaning to the intrinsic rate of natural increase. He extends the concept of \underline{r} to include a group within a population, an individual, or even a particular gene. Fisher also equates \underline{r} with "fitness" and holds the view that natural selection continuously tends to increase \underline{r} . That natural selection tends to maximize \underline{r} is also regarded as axiomatic by Lotka (1925) and Birch (1960). This is not to be confused with reproductive capacity in the sense of fertility or birth rate. According to Lotka (1925), natural selection tends to optimize the birth rate, \underline{b} , not to maximize it. The value of a species' \underline{r} is considered as being repeatedly lowered by what Fisher calls the

"deterioration of the environment." He also states that any positive balance left over when the rate of decrease in \underline{r} due to the deterioration of the environment is subtracted from the rate of increase in \underline{r} due to natural selection results in an increase in the population size.

The fact that \underline{r} is correlated with population size has special significance according to Andrewartha and Birch (1954). These authors believe that the distribution and abundance of animals are determined largely by the effects of the environment on \underline{r} . That is, a species will be found in greatest abundance where its \underline{r} is highest and that among potential competitors, the species that dominates will be the one with the highest \underline{r} .

In view of the importance of \underline{r} in population and community ecology, Cole (1954) made an analysis of the way the value of \underline{r} is influenced by various patterns of reproductive life-history. Among other things, Cole brings out clearly the extreme sensitivity of \underline{r} to age at first reproduction and the rapidly decreasing contribution to \underline{r} of offspring from parents of increasing age.

Lotka (1925) has derived an equation which defines \underline{r} in terms of the age-specific birth and death rates of the individuals in a population. He did this primarily to obtain a "true" rate of potential increase - one which is not biased by the prevailing age-distribution. He was able to show that any growing population is tending toward a definable "stable age-distribution." This is the age-distribution that the population would assume if the observed pattern of age-specific birth and death rates were to continue. The first actual calculation of an \underline{r} from life table data was made by Dublin and Lotka (1925) for

the human population of the United States in 1920. Since that time, this index has been utilized extensively in the field of demography.

The first calculation of an \underline{r} based on Lotka's equation for an animal other than man was made by Leslie and Ranson (1940) for the vole, Microtus agrestis. Leslie (1945) has also made calculations of \underline{r} for a rat, Rattus norvegicus, and Leslie and Park (1949) did them for a beetle, Tribolium castaneum.

However, it was Birch (1948) who first showed the usefulness of determinations of \underline{r} in experimental ecology. Before Lotka's introduction of an accurate method, values of \underline{r} were estimated by fitting the logistic equation to population growth curves. This method of determining \underline{r} and the logistic equation itself are subject to several valid objections (Birch, 1953b).

The intrinsic rate of natural increase may be used to compare a single species' capacity for increase under different conditions, or it may be used to compare this capacity in several species under the same or comparable conditions (Evans and Smith, 1952; Smith, 1954).

Birch (1953a; 1953b) has made an extensive study of the influence of temperature, humidity, and type of food on the value of \underline{r} in three species of grain beetles. He also found a correlation between \underline{r} 's determined from age-specific birth and death rates and \underline{r} 's determined by fitting the logistic equation to the observed population growth-form. In these three species, at least, Birch (1953c) found that the outcome of competition between any two of the species could be predicted strictly from the values of \underline{r} for the prevailing conditions of temperature and type of food. In all cases, only the species with the higher \underline{r} ultimately survived.

The complete list of r values to date consists of those for a few small rodents (Leslie and Ranson, 1940; Leslie, 1945; Leslie et al., 1952; Oliff, 1953), several species of terrestrial insects (Birch, 1948; Birch, 1953a; Birch, 1960; Evans and Smith, 1952; Howe, 1953; Leslie and Park, 1949), and only two aquatic animals (Dewitt, 1954; Frank et al., 1957). In spite of the ecological significance of r , the only studies made prior to the present one expressly to analyze the influence of environmental factors on r have been those of Birch (1953a) and Frank, et al., (1957).

The experimental study of radiation effects on properties of populations and communities is poorly represented in comparison with the number of studies that have been made on individual attributes. Among the latter should be included age-specific mortality rates and fecundity or fertility rates, longevity, and all kinds of "physiological" phenomena. On the other hand, there are several group-attributes which are unique to populations which have hardly been studied at all. These include an age-distribution, a population birth rate, a population death rate, intrinsic rate of natural increase, growth-form, average density, variability, mutation rate, and others.

There have been numerous studies of the effects of radiation on age-specific mortality, for which excellent reviews are available (Comfort, 1959; Strehler, 1959). Several investigations have been concerned primarily with the effects on age-specific fecundity and fertility (Carter et al., 1954; Casarett, 1956; Foster et al., 1949; Grosch and Smith, 1957; Park et al., 1958; Rugh and Clugston, 1955; Blair, 1958; McCarley, 1959; and others).

METHODS

Culture

Food organism: Chlamydomonas reinhardi was grown on sterile agar plates according to the method given by Richman (1958), except that soil extract was omitted, and shredded, rather than granulated, agar was used.

Experimental animals: Daphnia pulex were maintained at a constant average numerical density of one individual per 2 ml. of culture medium. The culture medium was renewed daily and consisted of natural spring water (purchased from the Arbor Springs Water Company) containing 100,000 Chlamydomonas cells per ml. This concentration was prepared by the method given by Frank et al. (1957), except that the algal cultures were from two to four weeks old when used, rather than from three to six weeks old. All the experimental animals were maintained at a constant temperature of $24 \pm 1^\circ\text{C}$. throughout life. They were also kept in constant darkness, except for the five hours each day required for routine handling. Each of the 21 populations studied was contained in a 125-ml. Pyrex beaker, and each beaker was replaced daily with a clean one. The cleaning of beakers consisted of scrubbing them with an artificial sponge under hot tap water while wearing rubber gloves, followed by two rinses in hot tap water and three final rinses with de-ionized water. The beakers were then placed upside down on a clean towel and allowed to dry. No soap or detergent was used.

Experimental Design and Routine Procedures

Method of irradiation: The different radiation intensities were obtained by placing the beakers containing the populations at different distances from a 5000-curie Co-60 source. The high radiation intensities produced by this source made it necessary to shield the populations with lead. Three control populations were kept in a lead-walled box in a "U"-shaped passage-way leading into the irradiation room. This box was not directly in view of the source. In the irradiation room proper, there were six other lead boxes having the shape of a cube eight inches to a side. The front wall of these boxes (the wall facing the source) was 7 cm. in thickness. The interior of these boxes provided environments whose radiation levels ranged from 20 to 75 roentgens per hour, depending (primarily) on the distance of each box from the source. Three beaker positions were located just inside the front wall of each box. Each population in a given box was kept in the same position (designated I, II, or III) throughout the period of study. The seven lead boxes, starting from the one closest to the source to the one containing the controls, are designated A through G, respectively.

Initiation of experiment: Fifty newborn daphnids (0-1 day old) were placed into each of 21 culture beakers, and culture medium was added to bring the volume to 100 ml. The order, or sequence, in which the different beakers were filled was arranged to preclude bias. The daphnids were taken from a finger-bowl containing a large excess over the total number (1050) required to initiate the experiment. The contents of the bowl were stirred each time before transferring an eye-dropper full of daphnids to depressions in a porcelain spot-test plate for counting. Personal selection was avoided by not looking

directly into the bowl when taking the daphnids up with the eye-dropper. This and all subsequent daily routine handling of the animals was done in a room whose temperature was matched with that of the Co-60 irradiation room.

After the beakers were filled they were taken to the irradiation room and placed in their designated positions in the lead boxes. Aluminum foil was used to keep dust and light out of the boxes.

Daily routine: The time registered on the "running time meter" of the Co-60 source was recorded upon removing the populations from the irradiation room, as well as when returning them. The meter registers the accumulated "up-time" of the source, or the time that the source is in the "up" position in the middle of the room. The populations were irradiated for an average of about 19 hours each day. The remaining time was taken up mostly by the daily routine handling of the animals, but some "down-time" was due to the use of the source by other investigators.

The daily handling of the animals consisted mainly of counting the survivors of the original group, separating and counting of the offspring, and transferring the survivors to a clean beaker containing fresh culture medium. All of the dead animals, offspring, and shed exoskeletons were preserved in formalin and stored in vials.

In separating the young from the adults, use was made of a convenient difference in their reaction to light. When the entire contents of a beaker were emptied into a petri dish, most of the young and adults accumulated on opposite sides of the dish. No nets were used. The complete separation of the young from the adults was accomplished with an eye-dropper while counting the adults in the depressions

of a spot-test plate. The newborn and young animals were counted with the aid of a large magnifying glass and a hand tally device.

The volume of fresh culture medium to which the survivors were returned depended upon their number. Two ml. were added per survivor, keeping the numerical density constant. Thus, each individual in all 21 populations always had potentially the same amount of food supplied to it each day, namely, 200,000 Chlamydomonas cells.

The order in which the different populations were handled was occasionally changed, but it was always a sequence designed to preclude bias. An example of the order in which the different populations were counted is:

	Position			
Box	I	II	III	row totals
A	1	12	20	33
B	21	9	3	33
C	13	5	15	33
D	8	18	7	33
E	14	2	17	33
F	4	19	10	33
G	16	11	6	33
column totals	77	76	78	

After counting and separating young and adults, and transferring the survivors to clean beakers with fresh culture medium, some individuals were placed on a glass slide in order to count eggs in their brood chambers and to measure their widths and lengths. Their

longest dimension stemming from the base of the spine was measured with an ocular micrometer in a compound microscope. Their maximum width perpendicular to this was also measured. For the first ten days, two individuals from each of the 21 beakers were measured. Starting on the eleventh day all of the individuals of only one of the beakers were measured on a given day. The order in which the individuals from different populations were measured was, so far as possible, the same as the example given above. The individual's sex and number of eggs or embryos in its brood chamber were also recorded.

Life Table Data

The age-specific rates of survival, l_x , and fertility, m_x , (where x = age in days) were calculated from primary cohort data as follows:

$$l_x = \frac{\text{number of females surviving to age } x}{\text{number of females present at age zero}}$$

$$m_x = \frac{\text{total number of female births between } x-0.5 \text{ and } x+0.5}{\text{average number of female survivors present between } x-0.5 \text{ and } x+0.5}$$

The equations by which the intrinsic rate of natural increase, stable age-distribution, population birth rate, population death rate, and average life-span were calculated from each of the 21 sets of life table data are developed and listed in Appendix A.

Dosimetry

Dose rates were measured with a Victoreen thimble chamber with a scale range of 0 to 100 roentgens. The chamber had thick walls, specially designed for measurement of high-energy gamma radiation. The

instrument is rated as having an accuracy of ± 5 per cent. Three readings were made for each of the three positions in each of the six lead boxes that was directly exposed to the source in the irradiation room. All of these readings were made with the "thimble" immersed in water contained in the culture beakers. Two readings were also taken in the control positions using a similar chamber, but one having a scale range of only 0 to 2.5 roentgens.

RESULTS

Dose Rates

Actual measurements of the dose rates in beakers of water at each position in each box in the irradiation room showed that the intensities of the radiation at the different positions within a given box were not the same (Table I). Each position in each box was compared with the other two positions (lumped together) by means of the Mann-Whitney "U" test (Seigel, 1956). In boxes B, C, and E no significant differences were found, but positions AI, DII, and FII were found to differ significantly ($P < 0.05$) from the other two positions in their respective boxes.

Effects of Radiation on Population Attributes

Intrinsic rate of natural increase: Since each population in a given box was not exposed to the same dose rate, the calculated population parameters for each population have been treated separately as single samples for each dose rate (Table II).

The intrinsic rate of natural increase, \underline{r} , decreases with increasing dose rate in a non-linear relationship (Figure 1). The slope of the curve (negative) increases continuously with increasing dose rate. The complete range of radiation intensities within which \underline{r} is positive is included here. This is the range of radiation intensities within which Daphnia pulex has some capacity to increase -- at a rate related to radiation intensity. Dose rates much beyond this range are of little ecological significance, since a population would not be able to exist under these conditions -- at least not for long.

Table I

Exposure Dose Rate Determinations, in Roentgens per Hour,
for Each Position in Each Box in Irradiation Room

Position	Measurement Number			Mean
	1	2	3	
AI	74.6	77.2	76.0	75.93
AII	72.0	71.2	68.9	70.70
AIII	68.3	72.4	68.3	69.66
BI	61.1	73.9	67.6	67.53
BII	61.5	65.3	65.7	64.16
BIII	60.2	77.4	64.0	67.20
CI	51.0	56.1	54.1	53.73
CII	50.9	52.6	53.2	52.23
CIII	52.2	54.3	55.9	54.13
DI	47.0	47.8	49.0	47.94
DII	43.8	45.5	46.5	45.26
DIII	49.0	49.7	46.7	48.46
EI	36.4	38.1	36.7	37.06
EII	36.9	39.1	39.0	38.33
EIII	38.0	38.8	38.1	38.30
FI	23.3	24.7	24.1	24.03
FII	22.0	22.9	23.5	22.80
FIII	24.5	28.4	23.6	25.50

Table II

Population Birth Rate, \underline{b} , Death Rate, \underline{d} , and Intrinsic Rate of Natural Increase, \underline{r} , for 21 Populations of Daphnia pulex Exposed to Different Intensities of Continuous Gamma Radiation

Population Exposed to Mean Dose Rate (Roentgens per Hour) of:	\underline{r}	\underline{b}	\underline{d}
0(control no. 1)	0.322	0.327	0.003
0(control no. 2)	0.334	0.335	0.001
0(control no. 3)	0.316	0.325	0.010
22.80	0.304	0.300	0.006
24.03	0.334	0.334	0.001
25.50	0.269	0.278	0.008
37.06	0.272	0.278	0.006
38.30	0.202	0.212	0.009
38.33	0.281	0.282	0.001
45.26	0.225	0.233	0.008
47.94	0.196	0.201	0.008
48.46	0.140	0.153	0.012
52.23	0.136	0.150	0.014
53.73	0.121	0.135	0.013
54.13	0.092	0.115	0.023
64.16	0.016	0.063	0.047
67.20	-0.021	0.045	0.070
67.53	0.015	0.061	0.046
69.66	0.043	0.080	0.033
70.70	0.090	0.099	0.020
75.93	-0.080	0.025	0.105

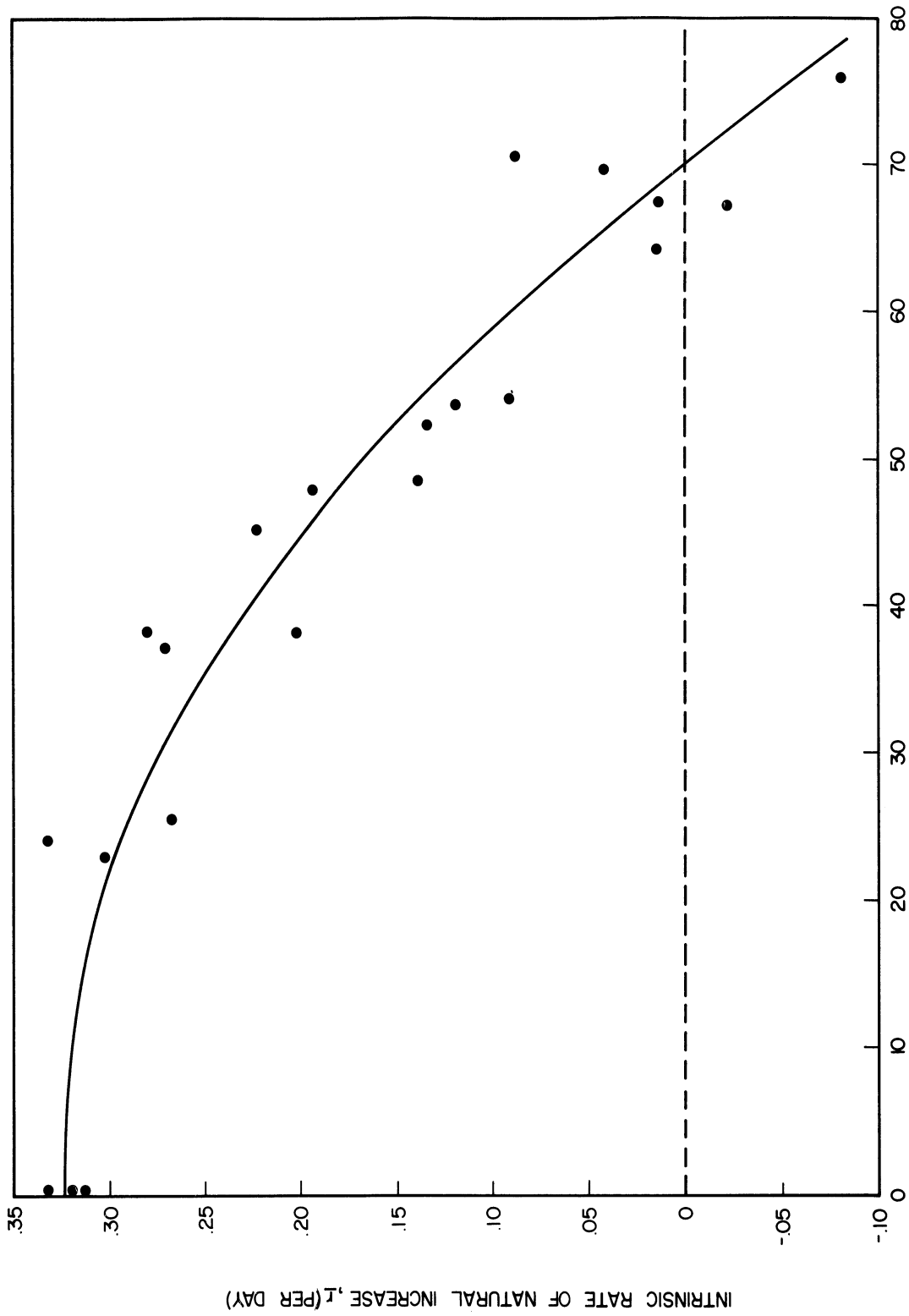


Figure 1. The effects of continuous gamma radiation on the intrinsic rate of natural increase of Daphnia pulex.

However, that dose rate of continuous radiation at which a species' \underline{r} is reduced to zero is of special interest. In Daphnia pulex this is about 70 roentgens per hour.

The results do not indicate a true threshold for the effects of continuous radiation on \underline{r} , although the effect of a given increase in dose rate is much smaller in the low range than in the high range (Figure 1).

The effect of radiation on \underline{r} is the product of many intermediary processes. The processes immediately underlying \underline{r} are the population birth rate, \underline{b} , and the population death rate, \underline{d} .

Population birth rate and death rate: The effects of continuous radiation on these parameters were calculated independently and plotted against dose rate (Figure 2). Since $\underline{r} = \underline{b} - \underline{d}$, it is now clear that the reduction of \underline{r} with increasing dose rate is due almost entirely to changes in the birth rate, \underline{b} . In the range from zero to 50 roentgens per hour, the birth rate falls sharply, while the death rate is barely affected (Figure 2).

The slope of the curve for either \underline{b} or \underline{d} at any given dose rate is taken as a measure of the sensitivity of these parameters to continuous radiation of that intensity. Thus, at one roentgen per hour, the sensitivity of \underline{b} is estimated to be -13×10^{-5} per day per roentgen per hour (estimated from an approximately linear relationship obtained by plotting \underline{b} against the square of the dose rate). The corresponding sensitivity of \underline{d} is only 8×10^{-6} per day per roentgen per hour (Figure 2). The relative sensitivity of the birth rate, as compared with the death rate, can be measured by the ratio of the sensitivity of \underline{b} to the sensitivity of \underline{d} . Thus, at one roentgen per hour, the birth rate is 16 times

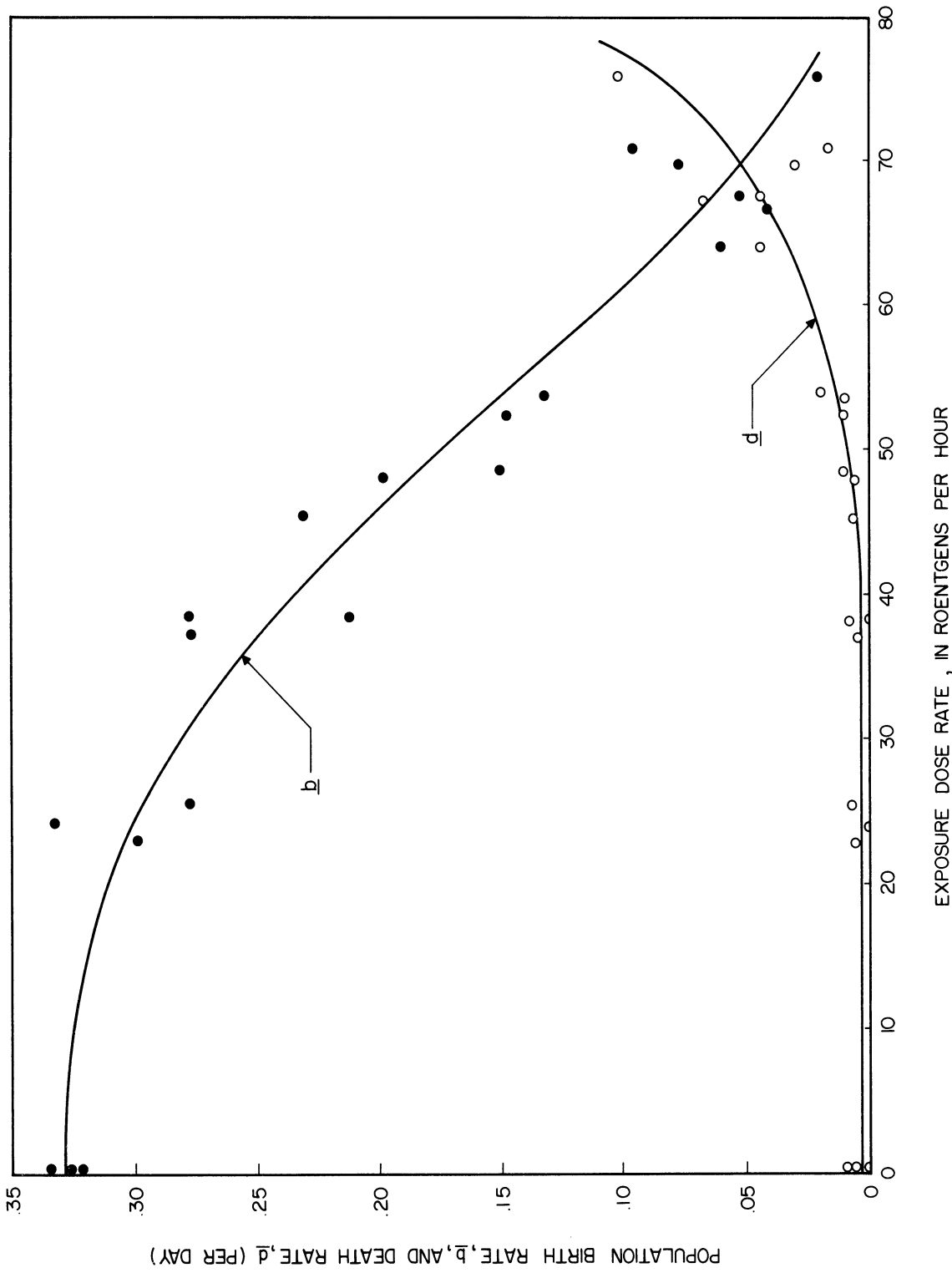


Figure 2. The effects of continuous gamma radiation on the population birth rate (dots) and the population death rate (circles) of Daphnia pulex.

more sensitive than the death rate. These relationships are different at higher dose rates. At 37 roentgens per hour, the birth rate is estimated to be 600 times more sensitive than the death rate.

The sensitivity of the birth rate, \underline{b} can be translated into more concrete terms. A sensitivity of -13×10^{-5} per day per roentgen per hour means that, for an increase from zero to one roentgen per hour, there would be approximately 13 fewer young born per day (instantaneous rate) per 100,000 individuals in the population. The corresponding finite rate for an interval of one day may be calculated from the equation, $B = e^{\underline{b}} - 1$; thus, $e^{-13 \times 10^{-5}} - 1 = -12 \times 10^{-5}$. The average birth rate, \underline{b} , at one roentgen per hour is approximately 0.330 per day -- from which B equals 0.391 per day or 39,100 offspring per 100,000 individuals in the population per day. The proportion of young not born due to radiation at one roentgen per hour is, therefore, 12 out of 39,100 or one out of 3,260.

The curve for \underline{b} as a function of dose rate exhibits an inflection near 55 roentgens per hour, and the curve becomes concave upward at higher dose rates (Figure 2). From a pilot experiment it is known that, \underline{b} , equals zero at and above approximately 140 roentgens per hour. This also indicates that \underline{r} equals minus infinity somewhere between 80 and 140 roentgens per hour and all higher intensities.

The death rate (Figure 2) begins to rise sharply at 50 roentgens per hour. It can approach plus infinity as a limit.

Stable age-distribution: Although the stable age-distributions for each of the 21 populations were calculated (Appendix B), only four have been illustrated (Figure 3). These four age-distributions illustrate

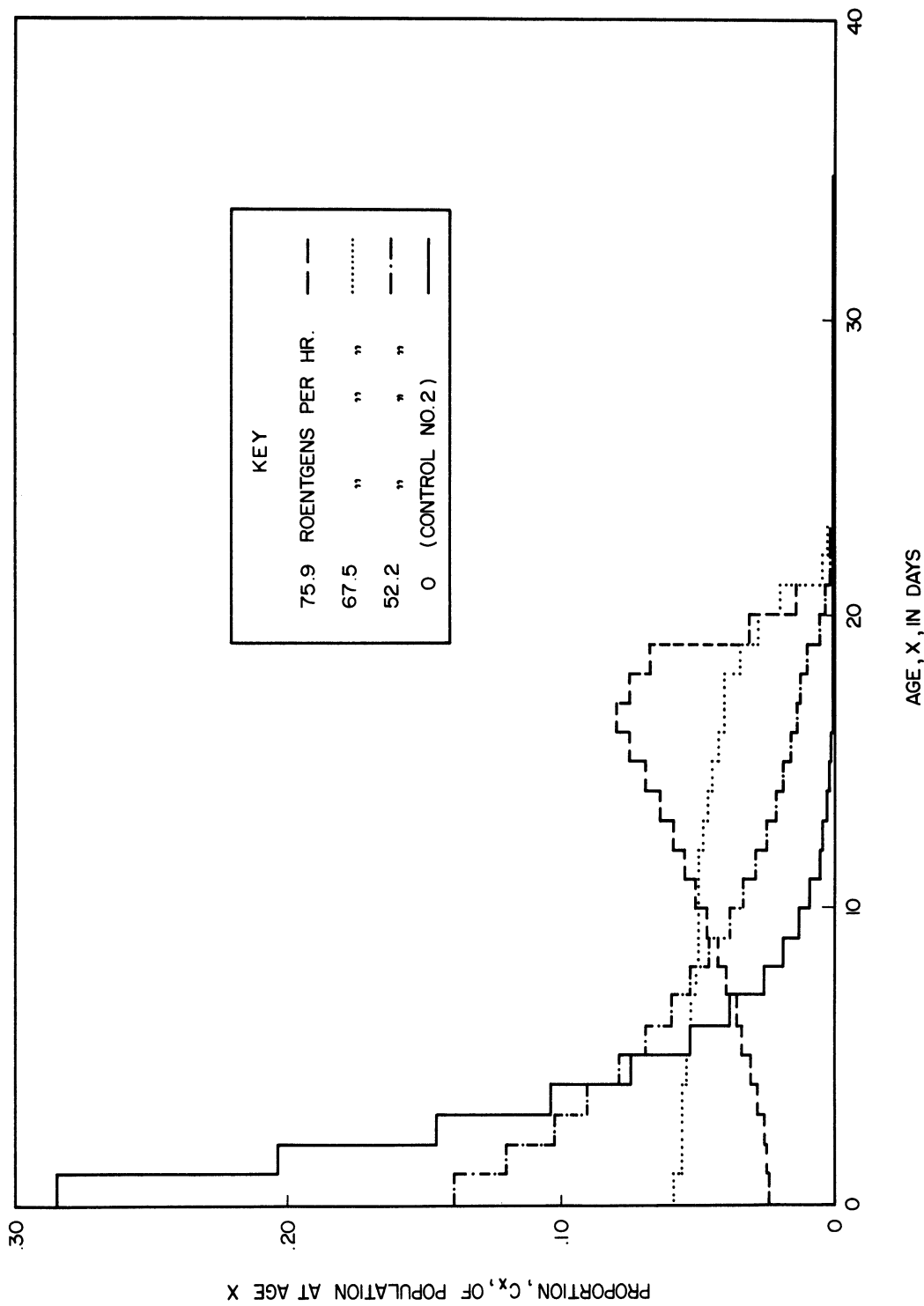


Figure 3. The effects of continuous gamma radiation on the stable age-distribution of Daphnia pulex. Out of the 21 populations, only four representative ones are illustrated. The dose rates to which each was exposed is indicated by the key.

the trend with increasing dose rate. The remaining age-distributions are intermediate to those illustrated for control population number two and the population at 75.9 roentgens per hour (Figure 3).

The radiation causes much more variation in the proportions of the youngest and the oldest individuals than in the proportion of individuals six to eight days old. The proportion of newborn decreases, while the proportion of individuals 10 to 19 days of age increases, with increasing dose rate.

In the control population, the proportion of individuals of a given age drops off rapidly and continuously with increasing age. The proportion of individuals older than 10 days (0.0347) is negligible in comparison with the proportion of younger individuals, although a ~~rare~~ individual may live as long as 35 days.

In the population receiving 52.2 roentgens per hour, the proportion of individuals more than 10 days old is still a small fraction of the total, but it is much larger than the corresponding proportion in the controls. The proportion of individuals of a given age drops continuously (but not as rapidly as the controls) with ~~in~~creasing age.

All populations receiving between zero and 52.2 roentgens per hour have stable age-distributions which are intermediate to the two described above (Appendix B).

In the population receiving 67.5 roentgens per hour, r is close to zero but still slightly positive. This population has an associated stable age-distribution which has essentially the same form as the survival curve for individuals in this population. Here, the proportion of individuals in the population older than 10 days is relatively large, and the proportion of individuals drops very gradually with increasing age.

In the population receiving 75.9 roentgens per hour, \underline{r} is negative; this is a population decreasing in size. The stable age-distribution of this population is composed of a comparatively small number of newborn, and the proportion increases with increasing age up to a maximum in the age category $x = 16$ (16 to 17 days of age). The proportion of individuals older than this drops rapidly with increasing age, and none lives longer than 22 days.

An important point concerning the stable age-distributions is that they partly determine the relative contribution of individuals of different ages to the birth rate, \underline{b} , and the death rate, \underline{d} . For instance, the individuals in the control population younger than 10 days carry much more weight in determining \underline{b} and \underline{d} than older individuals simply because the latter form a negligible proportion of the total population. On the other hand, in a nearly stationary population, such as the one receiving 67.5 roentgens per hour, the individuals older than 10 days may have an important influence on the population birth rate and death rate.

Effects of Radiation on Individual Attributes

Age-specific survival: For the same reasons given above, the age-specific survival rates, l_x , for the different populations have not been lumped into groups. The survivorship of each population is given together with its own dose rate in Appendix C.

The age-specific survival rates in every population are equal to or greater than 0.90 throughout the first 16 days. On the other hand, survivorship is less than 0.90 in every population after 22 days. In all cases, survivorship declines very gradually to the

0.90 value, but rapidly thereafter to zero. The age at which survivorship begins to decline rapidly is 17 days at the highest dose rates and increases to 22 days in the control populations. There are clearly no significant differences in survival among any of the 21 populations during the first 16 days (Appendix C). All of the populations exhibited almost ideal "rectangular" survival curves (Figure 4).

Life-span: Mainly because of differences in survivorship after 16 days (Appendix C), the average life-span, or life expectancy at birth, e_0 , is shortened with increasing dose rate (Figure 5). The mean life expectancy at birth for the three experimental populations exposed to the lowest dose rates is higher than the mean for the controls, but the difference is not significant ($p = 0.50$) according to the Mann-Whitney "U" test (Siegel, 1956). The highest value of e_0 , 27.04, appears to be aberrant. Although a real increase in life-span is possible at low dose rates, I prefer to interpret the data as indicated by the curve (Figure 5).

Age-specific fertility: The age at which reproduction begins is not affected by the radiation. Reproduction begins at an age of five days. Very few young were found on $x = 5$, but all populations had released most of the first brood by $x = 6$. Thus, the age-specific fertility rates in all populations tend to reach a peak synchronously at $x = 5.5$ (Appendix D).

Contrary to the result for early survival rates, the fertility rates are immediately affected by the radiation in the first brood. The Spearman rank correlation coefficient calculated from the m_x data for $x = 5.5$ was -0.8867. This has an associated probability of less than

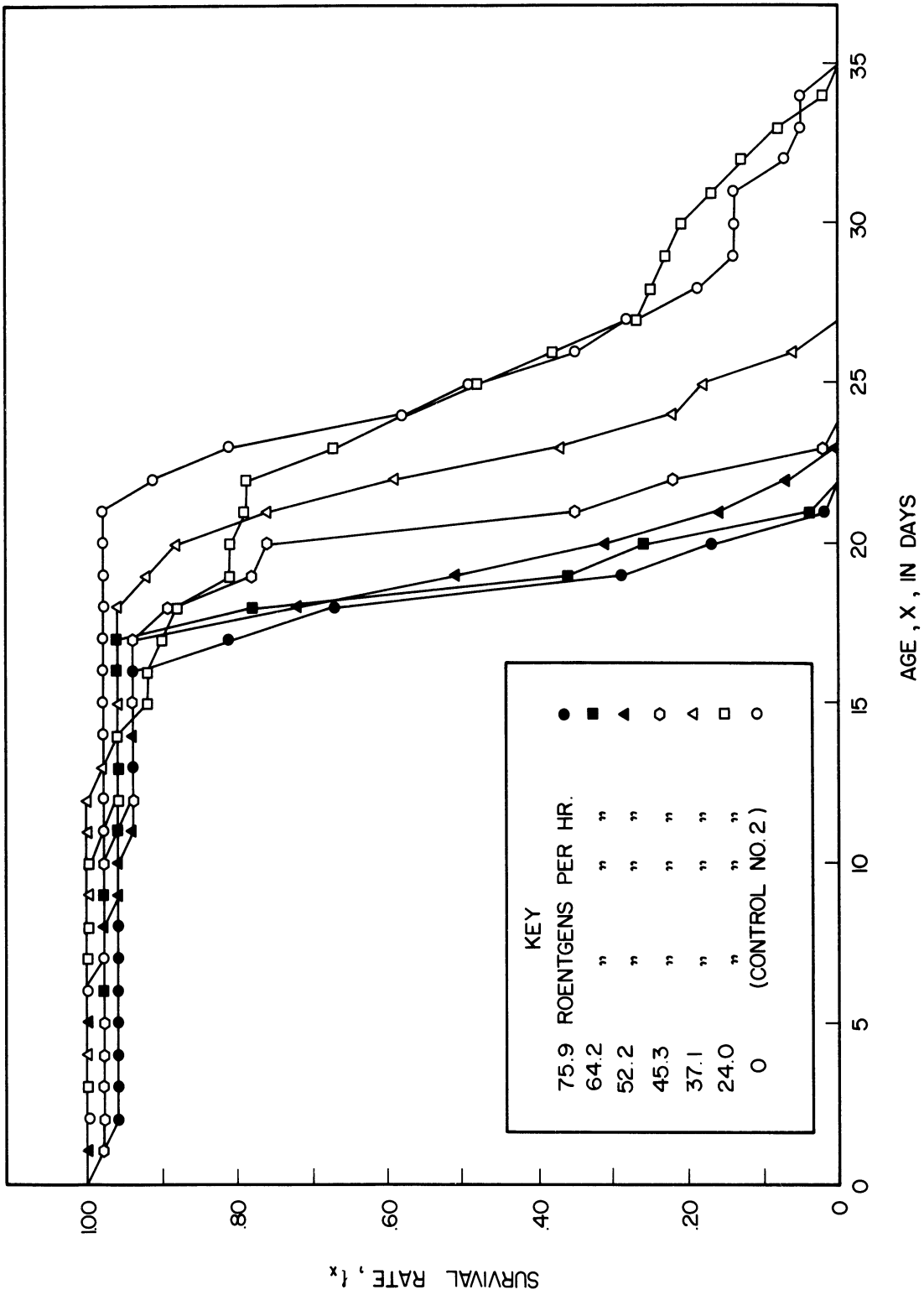


Figure 4. Seven representative survival curves for *Daphnia pulex* exposed to different dose rates (indicated by key) of continuous gamma radiation.

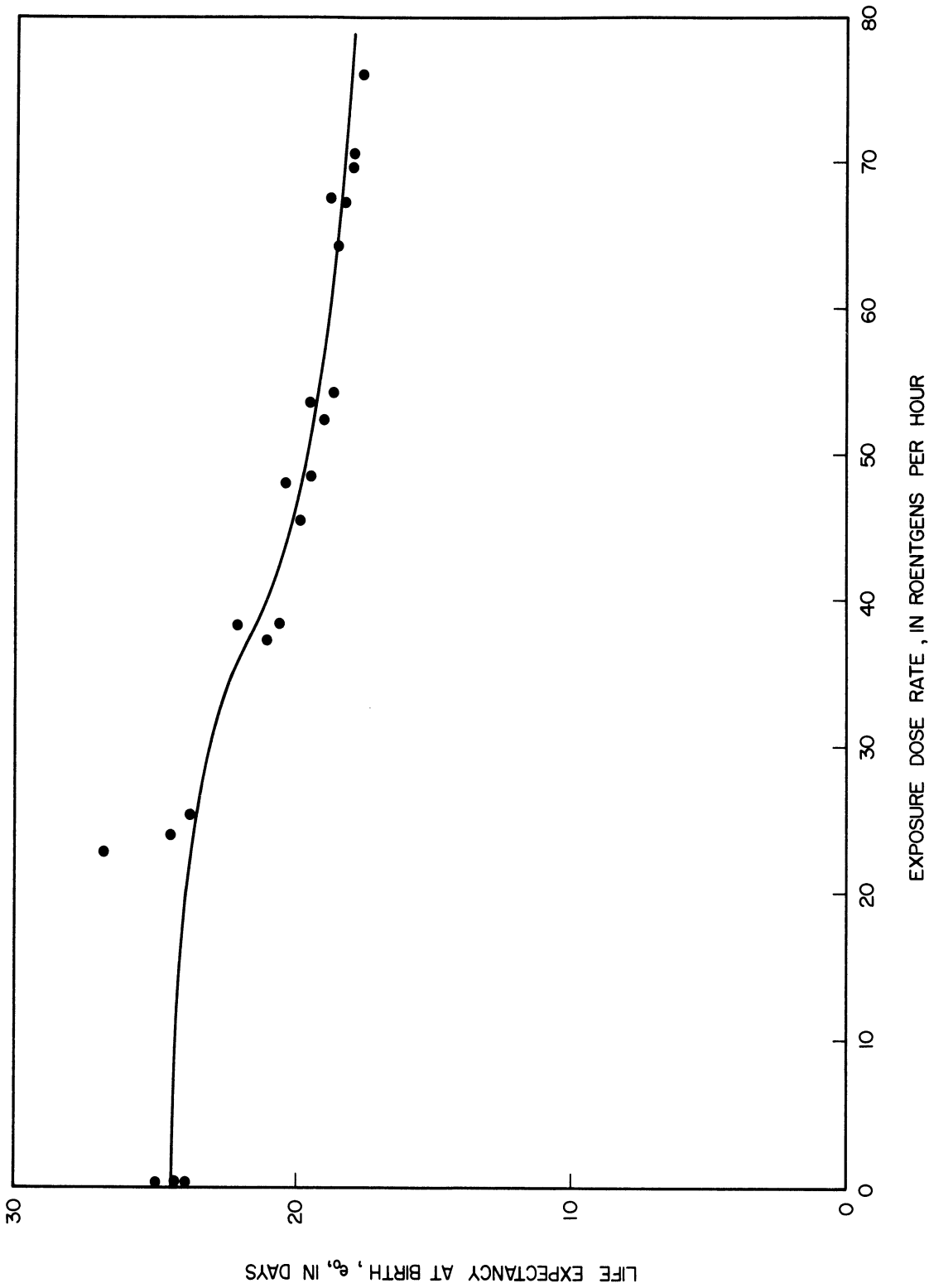


Figure 5. The effects of continuous gamma radiation on the life expectancy at birth, e_0 , or average life span (in days), of Daphnia pulex.

0.01 (Siegel, 1956). Thus, there is a high degree of negative correlation between fertility and dose rate from the very beginning of reproduction.

The age-specific fertility tends to fluctuate synchronously in succeeding broods, with a second peak at $x = 7.5$ and a third at $x = 9.5$ to 10.5 (Appendix D). A general decline in reproductive vigor commences earlier and reaches zero sooner with increasing dose rate.

The age at which permanent and complete sterility occurs is also correlated with dose rate. A "sterilization dose" was calculated for each population subjected to continuous irradiation (excluding the controls). This accumulated dose was obtained by multiplying the age at which permanent sterility ensued by 19 (hours of radiation per day) and the dose rate for each population. These values, when applied to the Kolmogorov-Smirnov one-sample test (Siegel, 1956), give a maximum deviation, "D", of 0.037. This value has a probability exceeding 0.20; hence, it is concluded that there are no significant differences in the sterilization dose for the different dose rates in the range covered here. The average accumulated dose from continuous radiation required to cause complete and permanent sterility (i.e., the sterilization dose) is 10,472 roentgens.

Individual growth: A somewhat surprising result is that the individual growth (in length) increases with increasing dose rate (Figure 6). This is also true for growth in width, and there is no noticeable change in shape. The average lengths for each day after $x = 5$ were grouped into high (boxes A and B), medium (boxes C, D and E), and low (boxes F and G) radiation levels and tested for differences using an extension of the median test (Siegel, 1956). The calculated Chi-square

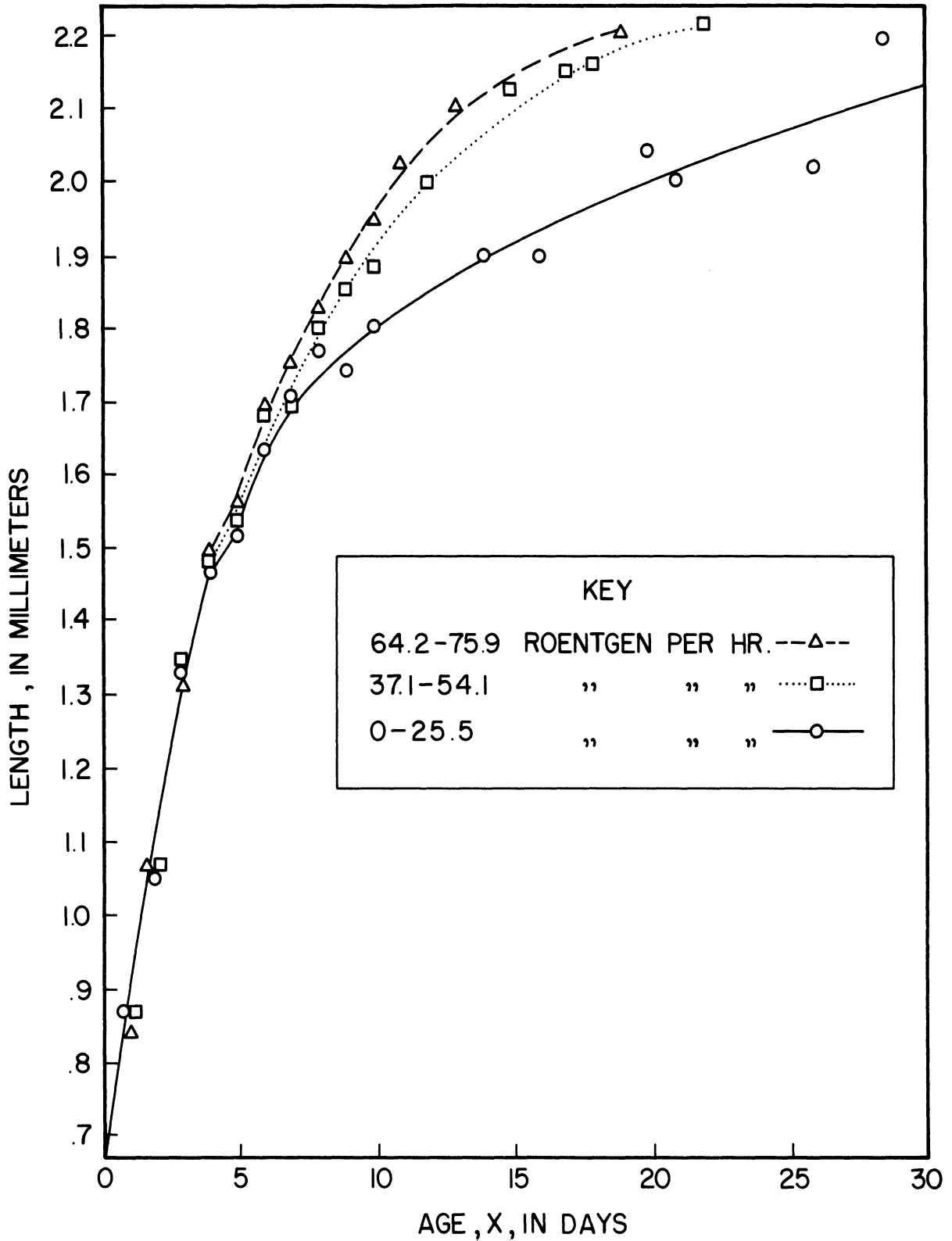


Figure 6. Individual growth in length (in millimeters) of *Daphnia pulex* exposed to "high" (triangles), "medium" (squares), and "low" (circles) intensity ranges of continuous gamma radiation.

value is 23.7. The associated probability, with two degrees of freedom, is less than 0.001. The growth curves illustrated in Figure 6 represent the trends in average length for individuals in 21 populations grouped into high, medium, and low radiation levels, as explained above.

Individual growth continues throughout life at all radiation levels, although the average length of individuals after the age of 16 or 17 days increases only slightly. Molting also continues normally (apparently) at all radiation levels throughout life.

Feeding: For the first four or five days a decreasing concentration of algal cells remained in suspension after a day's period of feeding. After the fourth or fifth day, the culture medium was apparently cleared of algal cells every day at all radiation levels. A slight sediment of dark fecal matter was present at the bottom of the beakers at the end of the day. This was true for several days. However, on the sixteenth day, I first noticed that there was some algae remaining in some of the beakers. The amount of algae remaining in suspension (as judged by color and transparency) was correlated with dose rate. In the populations above 40 roentgens per hour there was an increasing amount of algae left in the beaker. The six populations below 40 roentgens per hour had cleared the medium of algal cells. The individuals at the highest dose rates had apparently lost the ability to feed because their guts were much lighter in color than the controls and the concentration of algae remaining in suspension at the end of the day appeared to be almost the same as that being supplied.

On the 21st day the filtering ability of four individuals from populations in box D was compared with the filtering ability of four in-

dividuals in the control populations. This was done by comparing pairs of individuals on a glass slide under a microscope. They were surrounded by a small amount of water containing a high concentration of algae. The control individuals were able to collect large masses of algae between the bases of their legs in a few seconds. Most of the accumulating mass was periodically kicked out by the post-abdomen, but some of it reached the mouth, where it was acted upon by the mandibles. The individuals from populations in box D, however, were able to collect very little or no algae between the bases of their legs. After the 27th day, all populations, including the controls, had at least a small amount of algae remaining in suspension at the end of a day.

Eggs and embryos: The reduction of fertility with increasing dose rate beginning with the first brood is due almost entirely to reduced fecundity (egg production) rather than to increasing pre-natal mortality of embryos. A very small proportion of moribund eggs and embryos was observed in the brood chambers during the early reproductive period, but this was not correlated with dose rate. The proportion of moribund eggs and embryos in the brood chambers increased with age at all radiation levels, including the controls. After the fertility rates had declined to zero, there were also no more eggs released into the brood chambers.

Miscellaneous observations: Other than an increase in size and a decrease in fertility with increasing dose rate, no visible differences were noted in microscopic examinations during the first 16 days. Later, however, certain progressive, age-correlated changes were noted. These changes were also correlated with dose rate, appearing

earlier at higher dose rates. These include shortening of the terminal spine, loss of small spines on the posterior ventral edge of the carapace, decreasing heart rate and swimming activity, and loss of body color. In addition, the ovaries lost their general dark color, became motley with black pigment spots, and decreased in size.

Observations on second generation: Out of 121 first brood offspring raised to maturity, there were no males. These individuals were from all radiation levels; therefore, it is concluded that the second generation, like the first, consisted entirely of females in every population.

One of these second-generation adults had an unusual head shape -- very similar to the "excavated-head" mutation described for Daphnia longispina (Banta, 1939; cf. Figure 81).

DISCUSSION

The most important conclusion in the present study is that the intrinsic rate of natural increase of Daphnia pulex is reduced by exposure to continuous ionizing radiation in proportion to its intensity. Since \underline{r} and the other population attributes in this study are calculated entirely from life table data for a single generation, an important limitation to this conclusion concerns the uncertainty of the relationship of \underline{r} as a function of dose rate in succeeding generations. It is generally accepted that any increase in ionizing radiation increases mutation rates proportionately. Therefore, the possible consequences of continuous, sub-lethal intensities of ionizing radiation in Daphnia pulex due to mutations or chromosome aberrations must be considered.

In the present study, all dominant mutations or chromosome aberrations in the gonial cells which lowered fecundity and fertility are accounted for in the results. On the other hand, all recessive mutations and any dominants which would not influence the offspring until some time after birth are not accounted for in the results. However, at least in Drosophila, dominant lethals pose a much greater threat to population survival than recessive lethals (Wallace and Dobzhansky, 1959). If dominant lethals pose the major mutagenic threat to a population, the dose rate which will just suffice to reduce \underline{r} to zero, or slightly below, can be determined by the approach employed in the present study with some confidence. In Drosophila melanogaster this dose rate amounts to slightly over 5000 roentgens per generation (Wallace and Dobzhansky, 1959). In Daphnia pulex, the length of a generation varies with dose rate; nevertheless, the dose rate which reduces \underline{r} to zero is

approximately 8000 roentgens per generation. According to this index, then, Drosophila melanogaster is slightly more sensitive to continuous gamma radiation than Daphnia pulex.

In nature, radiation intensities much lower than those sufficient to reduce \underline{r} to zero may still have significant effects! Referring again to the principle that a species' maximum \underline{r} is balanced against the average resistance of the environment, one can imagine that even the slightest lowering of \underline{r} may result in a subtle contraction of the species' range, or a reduction of its average abundance. A lowered \underline{r} may result in more frequent elimination by competitors, predators, or parasites.

Besides reducing a species' \underline{r} through mutagenic effects, an increase in radiation intensity can also operate as a selective agent. If differential fertility or life-span of individuals has a genetic basis, radiation can alter the gene pool through natural selection, and a more resistant population may be produced (Green, 1960). However, the mutagenic effects of radiation probably become quite significant at dose rates which produce negligible direct somatic effects.

The occurrence of parthenogenesis as well as sexual reproduction in natural populations of Daphnia pulex merits special attention with regard to the possible consequences of ionizing radiation. Under favorable conditions, reproduction may be exclusively by diploid, apomictic parthenogenesis, in which "meiosis is totally absent and consequently no new gene combinations are possible, the progeny being genotypically similar to the mother" (Suomalainen, 1950). In the Cladocera, Suomalainen lists several cytological studies reporting

apomictic parthenogenesis, including Daphnia pulex, D. magna, and Polyphe-
mus pediculus. During parthenogenetic reproduction, new genetic
variability arises only through gene mutation and chromosome aberrations.
Recessive mutations can only accumulate. Therefore, a long
period of parthenogenesis in a Daphnia population exposed to an un-
usually high radiation level, may result in the homologous chromosomes
becoming incompatible in subsequent meiosis or in new zygotic combina-
tions. Indeed, even under normal radiation levels, Banta (1939) demon-
strated the gradual loss of average reproductive fitness of sexually
produced individuals as compared with parthenogenetically produced in-
dividuals in succeeding generations. He attributes this to the progres-
sive accumulation of "spontaneous" recessive mutations during partheno-
genesis.

In nature, Cladocerans presumably derive a competitive advan-
tage through parthenogenesis, in being able to avoid competition for
food with males of their own species. Every individual consumes food
which will be used in the production of eggs. A further advantage is
possibly a greater efficiency of natural selection in producing a popu-
lation that is highly adapted to the prevailing environment. In the
spring, hatching of over-wintering, fertilized eggs occurs, producing
a wide assortment of clones available for natural selection. Intense
selection during population growth may result in an increasingly homo-
geneous array of genotypes. When conditions become unfavorable, as in
the fall, parthenogenetically produced males appear. Soon afterward,
the females produce haploid eggs by meiosis. The induction of sexual
reproduction is controlled by the environment (Banta, 1939). This

alternation of sexual reproduction with parthenogenesis is somewhat uncommon in the animal kingdom, and it may place Cladocera in an unusually critical position with respect to the consequences of increased radiation intensities.

In a study of the effects of numerical density in Daphnia pulex, Frank et al. (1957) found that the birth rate was much more labile than the death rate. The same relationship was found in the present study for the effects of radiation. However, the same relationship may not hold in other species, especially in slowly maturing, long-lived species with low intrinsic fertility rates. In species with this type of life-history pattern, the death rate may be more labile than the birth rate. This is pure speculation, but a point to consider if one wishes to extrapolate from one species to another.

In animals with life-history patterns similar to Daphnia pulex, the effects of continuous radiation on age-specific mortality rates or life-span will probably have little influence on population parameters if fertility rates are also being affected, especially when mortality rates are not affected during early reproduction. In Daphnia pulex, the radiation intensity must approach levels which threaten extinction of the population before the death rate shows any sign of radiation effects. The birth rate is much more sensitive than the death rate.

In Daphnia pulex, the proportion of offspring prevented from being born at a dose rate of one roentgen per hour was estimated to be one out of 3,260. Although this is based on growing populations with a high birth rate, the proportion at a given dose rate is probably independent of birth rate. If so, the same proportion will be prevented

from being born in steady-state populations exposed to one roentgen per hour.

The extent to which effects of radiation on age-specific fertility and mortality rates are reflected, respectively, in the population birth rate or the population death rate depends on the age-distribution in the population. In general, effects on a given age-specific fertility rate (or mortality rate) will influence the population birth rate (or death rate) only if that particular age group constitutes a relatively significant proportion of the population.

In the present study, the death rate begins to rise rapidly above 50 roentgens per hour. This is not due to a greater increase in mortality rates in this range compared to lower intensities, but rather to an increasing proportion of older individuals in these populations. The latter, in turn, is due to the greatly reduced intrinsic rate of natural increase. Most of the observed rise in the death rate beyond 50 roentgens per hour would have occurred even if mortality rates had not been affected at all.

The effects of acute and, to a lesser extent, chronic ionizing radiation on survival and mortality have been studied in many species, especially mammals (Blair, 1954; Boche, 1954; Lorenz, 1954). Among the arthropods, radiation-induced mortality has been studied extensively in insects. Very little is known, however, of the effects of radiation on mortality in Cladocera or other Crustacea. The brine shrimp, Artemia salina, has been studied by Bonham and Palumbo (1951) and also by Grosch and Erdman (1955). The latter authors conclude that the response of adult Artemia to acute lethal doses of x-radiation places them

near but below the holometabolous insects in radiation tolerance. Snider and Kersten (1935; Kersten and Snider, 1935) have studied the effects of acute lethal doses of soft x-rays on adult Daphnia magna. The exposure dose rates are not stated, but from the data presented, I estimate 140,000 roentgens to be the dose which killed 50 per cent within one minute. Following exposures to 200,000 roentgens, 100 per cent were dead within one minute. This agrees fairly well with the exposures of 150,000 to 200,000 roentgens found by Grosch and Erdman (1955) to be required to kill outright 100 per cent of the adult Artemia. In the present study, the intensities of the radiation to which Daphnia pulex were exposed were too low to cause early deaths, and it is not known what doses or dose rates would be necessary to do so.

Although the effects of continuous or chronic low-level radiation in mammals have been studied quite extensively, little information is available for invertebrates with which to compare my results for Daphnia pulex. However, the general nature of the results obtained in the present study concerning the effects of continuous, sub-lethal radiation on mortality and life-span do not appear to be in conflict with those obtained for other species.

The survival curves obtained in the present study approach the rectangular, "physiological" type (Comfort, 1956; Bodenheimer, 1938; Deevey, 1947). This is taken to indicate a high suitability of the culture conditions and routine handling as well as homogeneity among the individuals. An acceleration of mortality rates begins at a "specific age" (Comfort, 1956) which is related to dose rate, and almost all of the animals die soon thereafter.

The observed effects of the radiation on survival rates and life-span are taken as a measure of the effects on somatic cells and "physiological" functions of the individuals. The fact that fecundity and fertility disclosed large effects from the very beginning of adulthood while survival rates were not affected until late in life is interpreted as evidence that the reduction of fecundity and fertility was due to direct effects of the radiation on the cells of the gonads and not to physiological effects.

It is now generally accepted that the nucleus is more sensitive to radiation than the cytoplasm of a cell (Gray, 1959; Mole, 1959; Stapleton and Hollaender, 1958; and others). According to Puck (1960b), cells that have been irradiated with ten to 100 times the dose which will permanently "sterilize" them (i.e., prevent cell division) continue to exhibit essentially normal metabolism and growth. Such cells become extremely large before finally dying.

It is also widely held that radiation-induced sterility or reduced fecundity and fertility is mainly due to chromosome aberrations. These act as dominant lethals in the gametogenic stem-cells or, to a lesser extent, in the gametes which mature successfully (Muller, 1954; 1958; Wallace and Dobzhansky, 1959; Lea, 1956). The loss of reproductive ability in isolated human cells cultured in vitro is also attributed to one-hit or two-hit chromosome aberrations (Puck, 1958; 1960a). The number of one-hit aberrations is linearly proportional to dose and is independent of dose rate. The number of two-hit aberrations tends to be linearly proportional to the square of the dose, but usually somewhat less than the square, depending on the proportion

of breaks which reconstitute normally. The proportion of two-hit chromosome aberrations per unit dose are not independent of dose rate (Lea, 1956; Muller, 1954; 1958; Wallace and Dobzhansky, 1959).

In the present study, the fact that the proportion of offspring prevented from being born approximates a linear relationship with the square of the dose rate suggests that two-hit chromosome aberrations may be the major cause of reduced fecundity and fertility and ultimate sterility. The reduction of fertility in the present study is due mostly to reduced fecundity. It might be expected that, under continuous irradiation from birth, most of the eggs which manage to be produced will be free of dominant lethals because most of these would have resulted in mitotic arrest of the gametogenic cells which incurred them. However, following x-irradiation of young adults of two species of flour beetles (Tribolium confusum and T. castaneum) with acute doses of 2,000, 3,000, 4,000 and 5,000 roentgens (Park et al., 1958), the fecundity of the exposed and subsequent generations was reduced less than the hatchability of the eggs -- just the opposite relationship from what I found (for continuous radiation) in Daphnia pulex.

A radiation dose which will cause a reduction of fertility, even to the point of sterility, is usually much lower than that necessary to induce acute mortality (Lindquist, 1958). With continuous, low-level radiation, it is possible that a dose rate which will reduce the intrinsic rate of natural increase to zero is, likewise, much lower than that necessary to shorten life-span greatly. In the present study, the accumulated dose required to stop egg production (the sterilization dose)

is of the same order of magnitude as the accumulated dose per generation required to reduce \bar{r} to zero. When direct indications are not available, the sterilization dose for acute exposure may serve as an indication of the dose which, if accumulated prior to reproduction, will likely result in extinction of a population. However, for most radiation effects, except gene mutations, the accumulated dose for continuous or fractionated exposures is usually higher than the acute dose required to produce the same degree of effect. This is attributed to the action of repair during protracted doses.

The sterilization dose for continuous radiation in Daphnia pulex is approximately 10,500 roentgens and is independent of dose rate in the range from 20 to 75 roentgens per hour. This value agrees well with the "acute" sterilization dose in many insects, although the latter varies greatly among species (Jenkins, 1957). If the newborn or young adult Daphnia pulex in my study had been subjected to acute doses, the sterilization dose probably would have been much lower -- perhaps as low as the dose of 5000 roentgens which was found to produce sterility in adult Artemia salina by Grosch and Erdman (1955).

As these authors suggest, although the dose required to induce a given degree of acute mortality varies widely in the animal kingdom in relation to phylogenetic position, different species may be much more alike with respect to their response in fertility. As much as 3000 roentgens may also be required to insure complete sterility of female rats (Errera and Forssberg, 1960). On the other hand, females of certain strains of mice may be permanently sterilized by as little as 50 roentgens (Rugh and Wolff, 1956), and female grasshopper nymphs are sterilized by 350 to 420 roentgens (Tahmisian and Vogel, 1953).

In a study of the effects of x-rays on respiratory rate and growth in Simocephalus vetulus by Obreshkove and King (1932a; 1932b), individuals exposed to 9,840 roentgens shortly after birth were found six days later to be sterile although comparable in size to the controls, which were fertile. It is possible, however, that a lower dose would also have resulted in sterilization.

The effects of acute irradiation on mouse embryos depends on the stage of development exposed. In general, the pre-natal mortality caused by a given dose decreases as the embryo grows older. The one or two-celled stages are the most sensitive. Any exposure of the 12-hour mouse embryo (prior to the first cleavage) probably causes increased pre-natal mortality -- hence, reduced fertility. An exposure of five roentgens at this stage causes 11 per cent more deaths than among the controls, and 25 roentgens results in 38 per cent more deaths (Rugh and Grupp, 1959). Trout embryos are also more sensitive in the one-cell stage than at any later stage (Welandar, 1954).

In the present study, however, the dose accumulated during the one-cell stage, or even throughout development, would be very small in comparison with the dose accumulated by the gametogenic stem-cells of the ovaries. The eggs which are released into the brood chamber are produced by gonial cells still capable of dividing and are not likely to carry chromosome aberrations, since these tend to prevent division in cells which carry them (Muller, 1954; 1958; Puck, 1960a).

The increased growth in length at higher dose rates in the present study could be simply a result of a saving of assimilated energy and matter which in the more fertile individuals at lower radiation

intensities is spent in egg production. However, consumption of food by the greater number of newborn daphnids at the lower dose rates is possibly a contributing factor. When decreased fertility is due to direct, "physiological" effects, such as caused by differences in food supply, it is usually correlated with decreased, rather than increased, growth rates. In Daphnia pulex cultured at different levels of food supply per individual, growth rate and fertility are correlated positively with each other (Frank, et al., 1957; Richman, 1958).

As to the overall effects of increasing levels of radiation in biotic communities, at present there is almost no direct experimental work from which to draw conclusions. One example of how complex the situation becomes at the community level is provided by the reaction of a predator-prey system to acute doses of radiation. It has been found that with increasing dose, the number of prey increases, and the number of predators decreases (Auerbach, 1958). Auerbach suggests that the reason for this result lies in a differential radiation sensitivity between the predator and prey species. It is likely that for a given dose rate, the higher trophic levels in a biotic community will be directly affected more than the lower levels; nevertheless, the indirect radiation effects (through altered predation, competition, etc.) may become quite significant at all levels of a community. Indirect effects of radiation might occur in human populations as a result of changes in populations of disease vectors, parasites, pests, or even species in man's food chains.

Because of the special significance of a species' intrinsic rate of natural increase and the importance of this concept in population and community ecology, information is urgently needed on the effects of

radiation on r in species representing a variety of ecological niches. The methods and conceptual background employed in the present study of Daphnia pulex may be applied to species representing different trophic levels in terrestrial as well as aquatic food chains. The same approach may be taken in the study of the effects of internal radiations emitted from radio-isotopes in the food chains themselves.

Only after considerably more knowledge is gained through observations in nature and the experimental approach will it be possible to develop general principles of radiation ecology. Toward this end we have barely begun.

SUMMARY

1. Twenty-one cohorts of Daphnia pulex, each started with 50 newborn individuals, were exposed to different intensities of external gamma radiation throughout life. Numerical density in the cultures was maintained at 0.5 individual per ml.

2. The culture medium consisted of suspensions of Chlamydomonas reinhardi in spring water. The medium was replaced daily.

3. The number of survivors in each cohort and the number of offspring were determined daily. All of the newborn, dead individuals, and exoskeletons were removed. Samples of surviving individuals were temporarily removed daily for linear measurements, egg counts, and general observations.

4. The intrinsic rate of natural increase, \underline{r} , decreased continuously as a non-linear function of the radiation dose rate. At a dose rate of 70 roentgens per hour, \underline{r} equaled zero; at higher intensities it was negative.

5. The fall of \underline{r} with increasing dose rate was due almost entirely to a drop in the birth rate, \underline{b} , rather than a rise in the death rate, \underline{d} .

6. The death rate was not affected up to approximately 45 roentgens per hour. Beyond this it began to rise.

7. The effects of increasing radiation on the stable age-distributions associated with these populations were a decrease in the proportion of individuals in the youngest age groups and an increase in the proportion of "older" individuals. The proportion of

individuals in the intermediate age classes were relatively unaffected.

8. The rise in the death rate above 45 roentgens per hour was due to the increasing proportion of older individuals in these populations and was not due to a "threshold" for age-specific mortality effects or shortening of life-span.

9. The age-specific survival rates were all equal to or greater than 0.90 throughout early reproductive life, and there were no significant differences among the 21 populations. Almost all of the individuals of a given cohort tended to die simultaneously, indicating a sharply defined "specific age". This age was reduced at higher dose rates. The average life-span or life expectancy at birth, was not greatly shortened by the intensities of radiation employed in this study.

10. Age-specific fertility rates, in contrast to survival rates, were immediately affected commencing with the first brood. The age at which reproduction began, however, was not affected. Reproduction began (at 24°C.) between the fifth and sixth day of age. The reduction of fertility was due almost entirely to reduced fecundity rather than to increased pre-natal mortality.

11. Individual growth in length increased with increasing dose rate. This was interpreted as a result of a saving of assimilated energy and matter which at lower intensities was spent in egg production. However, consumption of food by the greater number of newborn daphnids at the lower dose rates was possibly a contributing factor.

12. Some of the possible consequences of increased mutation rates in Daphnia were discussed, and the present results for Daphnia pulex were compared with related information obtained by other investigators studying Drosophila melanogaster and other species including man.

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Appendix A

Treatment of Life Table Data

A population in which each generation follows the same age-specific rates of survival (l_x) and fertility (m_x) will ultimately assume a stable age-distribution. The associated birth rate, death rate, and intrinsic rate of natural increase of such a population will also become stable, or constant.

These population attributes may be calculated from life table data (age-specific fertility and survival rates) for a single, isolated generation (or cohort) followed throughout life. The equations by which the population attributes in the present study were calculated and their derivations which follow are based largely on unpublished material presented by Dr. F. E. Smith in ecology seminars. The first parameter to be calculated is the intrinsic rate of natural increase. Since it is easier to calculate finite rates than the corresponding instantaneous rates, the former are calculated first and later are converted into the corresponding instantaneous rates.

The finite intrinsic rate of natural increase, R , is the factor of multiplication in numbers of individuals per unit time and is equal to the natural anti-logarithm of r , the instantaneous intrinsic rate of natural increase:

$$R = e^r \quad (1)$$

The primary data needed for calculating R are the female births during each time interval per original female and the midpoints for each such interval. Since the births are counted at the end of a time interval, the time (x) column must be shifted to the midpoints.

When the $l_x m_x$ column (female births during each time interval per original female) for the midpoints of each time interval has been tabulated, that value of R is sought for which $\sum l_x m_x R^{-x} = 1.00$. The rationale for this is not complicated. If a "population table" is evolved (with the age categories in a horizontal row heading the columns and the time units in a vertical column heading the rows) any given age group will first appear at age zero in the left-hand column and will pass diagonally downward across the table as it ages. The numbers of individuals in the diagonals decrease according to the l_x data. Therefore, the development of the table may be started by entering the primary l_x data in a diagonal beginning with the l_0 age category (=1.00) in the upper left hand corner. Development may be continued by multiplying the number of individuals in each entry by the appropriate m_x factor to get the number of offspring produced by each group. The total number of offspring produced in each row in sequence is entered in the left-hand column for that row. The latter represents a new l_0 group from which a new diagonal can be produced by multiplying by the appropriate l_x factors. Eventually it will be found that each age category, as well as the total in each row, increases by the same, constant factor each time interval. This factor is R . The proportion in each age category remains constant and represents the stable age-distribution. However, it can now be shown that all this laborious work does not actually need to be done to determine R and the stable age-distribution.

The stable age-distribution is preserved if, in moving it to another row, each age category in it is corrected by the factor R for each time unit moved. To move a row upward one time unit, it is

divided by R (or multiplied by R^{-1}). Although the age-distribution in the rows changes considerably with time at first, the distributions along the diagonals do not. Hence, the l_x distribution beginning with 1.00 in the upper left-hand corner may be swung upward into a horizontal row representing the stable age-distribution by multiplying each l_x figure by R^{-x} (to bring it up to the first row).

The $l_x R^{-x}$ figures represent the stable age-distribution when the age zero category equals 1.00. The age zero category also represents the total number of offspring produced by all of the reproductive age groups in this row; hence:

$$\left. \begin{aligned} \sum l_x m_x R^{-x} &= 1.00 \\ \text{or,} \\ \sum l_x m_x e^{-rx} &= 1.00 \end{aligned} \right\} \quad (2)$$

Since the $l_x R^{-x}$ figures represent the stable age-distribution when the age zero category equals 1.00, the proportion, C_x , of individuals of age x , when the total population equals 1.00, is given by:

$$C_x = l_x R^{-x} / \sum l_x R^{-x} \quad (3)$$

The finite birth rate, B , may be defined as the ratio of births during a time interval to the total number of individuals present at the beginning of the time interval. The births are represented by the age zero category (1.00); therefore:

$$B = 1 / \sum l_x R^{-x} - 1 \quad (4)$$

The instantaneous birth rate, \underline{b} , equals the natural logarithm of $(1 + B)$:

$$e^{\underline{b}} = 1 + B \quad (5)$$

The finite death rate, D , is the ratio of the number of deaths

during a time interval to the total number of individuals present at the beginning of the time interval. It is easier to calculate its complement, the survival rate, $S = 1 - D$.

Any group of age x will survive to $x+1$ by the age-specific survival fraction, l_{x+1}/l_x . Hence, the survival of the $l_x R^{-x}$ age groups in the stable age distribution will survive one time unit later by the amount $l_x R^{-x}(l_{x+1}/l_x)$, or $l_{x+1} R^{-x}$. The overall population survival rate, S , is given by the ratio of the sums of these two:

$$S = \frac{\sum l_{x+1} R^{-x}}{\sum l_x R^{-x}} \quad (6)$$

The instantaneous death rate, \underline{d} , is the negative natural logarithm of S :

$$e^{-\underline{d}} = S \quad (7)$$

If calculations have been properly made, the finite and instantaneous rate parameters should satisfy the following equations:

$$R = (1 + B)(1 - D) \quad (8)$$

$$\underline{r} = \underline{b} - \underline{d} \quad (9)$$

In addition to the m_x and l_x data, another age-specific individual attribute to be considered is e_x , the life expectancy at age x . The average life-span, or mean number of animal-days lived, is equal to the life expectancy of an individual at birth, e_0 . This is calculated according to the following equation:

$$e_0 = \frac{(l_x + l_{x+1})/2}{l_0} \quad (10)$$

Appendix B

Stable Age-Distribution for 21 Populations of Daphnia pulex
Exposed to Different Intensities of Continuous Gamma Radiation.

(Each column in the body of the table refers to a single population.
The individual entries in each column are the proportion
of individuals of a given age, C_x , in that population.)

Age, x, in days	Population Exposed to Dose Rate (in roentgens per hour) of:				
	0 (control no. 1)	0 (control no. 2)	0 control no. 3	22.80	24.03
0-1	.279	.284	.278	.259	.284
1-2	.202	.204	.199	.187	.204
2-3	.143	.145	.142	.138	.146
3-4	.104	.104	.104	.109	.104
4-5	.075	.075	.076	.081	.075
5-6	.055	.054	.055	.060	.053
6-7	.040	.038	.040	.044	.038
7-8	.029	.027	.029	.032	.028
8-9	.021	.019	.021	.024	.020
9-10	.015	.014	.016	.018	.014
10-11	.011	.010	.011	.013	.010
11-12	.008	.007	.008	.009	.007
12-13	.006	.005	.006	.007	.005
13-14	.004	.004	.004	.005	.004
14-15	.003	.003	.003	.004	.003
15-16	.002	.002	.002	.003	.002
16-17	.002	.001	.002	.002	.001
17-18	.001	.001	.001	.002	.001
18-19	.001	.001	.001	.001	.001
19-20	.001	.001	.001	.001	.000+
20-21	.000+	.000+	.001	.001	.000+
21-22	.000+	.000+	.000+	.000+	.000+
22-23	.000+	.000+	.000+	.000+	.000+
23-24	.000+	.000+	.000+	.000+	.000+
24-25	.000+	.000+	.000+	.000+	.000+
25-26	.000+	.000+	.000+	.000+	.000+
26-27	.000+	.000+	.000+	.000+	.000+
27-28	.000+	.000+	.000+	.000+	.000+
28-29	.000+	.000+	.000+	.000+	.000+
29-30	.000+	.000+	.000+	.000+	.000+
30-31	.000+	.000+	.000+	.000+	.000+
31-32	.000+	.000+	.000+	.000+	.000+
32-33	.000+	.000+	.000	.000+	.000+
33-34	.000+	.000+		.000+	.000+
34-35	.000+	.000+		.000+	.000+
35-36	.000	.000		.000	.000

Appendix B (continued)

Stable Age-Distribution for 21 Populations of Daphnia pulex
Exposed to Different Intensities of Continuous Gamma Radiation

(Each column in the body of the table refers to a single population.
The individual entries in each column are the proportion
of individuals of a given age, C_x , in that population.)

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:				
	25.50	37.06	38.30	38.33	45.26
0-1	.242	.243	.191	.246	.208
1-2	.185	.181	.153	.186	.162
2-3	.136	.138	.125	.140	.130
3-4	.104	.105	.100	.106	.103
4-5	.079	.080	.082	.080	.083
5-6	.061	.061	.067	.060	.066
6-7	.046	.047	.054	.046	.053
7-8	.035	.036	.044	.034	.042
8-9	.027	.027	.036	.026	.033
9-10	.021	.021	.030	.020	.027
10-11	.016	.016	.024	.015	.021
11-12	.012	.012	.020	.011	.017
12-13	.009	.009	.016	.009	.013
13-14	.007	.007	.013	.006	.010
14-15	.005	.005	.011	.005	.008
15-16	.004	.004	.009	.004	.007
16-17	.003	.003	.007	.003	.005
17-18	.002	.002	.006	.002	.004
18-19	.002	.002	.005	.002	.003
19-20	.001	.001	.004	.001	.002
20-21	.001	.001	.003	.001	.002
21-22	.001	.001	.002	.001	.001
22-23	.000+	.000+	.001	.000+	.000+
23-24	.000+	.000+	.000+	.000+	.000+
24-25	.000+	.000+	.000+	.000+	.000
25-26	.000+	.000+	.000+	.000+	
26-27	.000+	.000	.000	.000+	
27-28	.000+			.000	
28-29	.000+				
29-30	.000+				
30-31	.000+				
31-32	.000+				
32-33	.000+				
33-34	.000+				
34-35	.000				

Appendix B (continued)

Stable Age-Distribution for 21 Populations of Daphnia pulex
Exposed to Different Intensities of Continuous Gamma Radiation

(Each column in the body of the table refers to a single population.
The individual entries in each column are the proportion
of individuals of a given age, C_x , in that population.)

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:				
	47.94	48.46	52.23	53.73	54.13
0-1	.184	.142	.139	.126	.109
1-2	.148	.123	.121	.111	.097
2-3	.122	.105	.104	.099	.087
3-4	.100	.091	.091	.087	.079
4-5	.182	.079	.079	.077	.072
5-6	.068	.069	.069	.069	.066
6-7	.056	.059	.060	.061	.060
7-8	.046	.051	.053	.054	.055
8-9	.038	.044	.046	.048	.050
9-10	.031	.039	.039	.041	.046
10-11	.026	.033	.034	.037	.042
11-12	.021	.029	.029	.032	.038
12-13	.017	.025	.026	.029	.035
13-14	.014	.022	.022	.025	.031
14-15	.012	.019	.020	.023	.028
15-16	.010	.017	.017	.020	.026
16-17	.008	.014	.015	.018	.023
17-18	.006	.013	.013	.015	.021
18-19	.005	.011	.010	.012	.017
19-20	.004	.008	.006	.009	.013
20-21	.003	.005	.004	.006	.005
21-22	.002	.001	.002	.002	.002
22-23	.001	.001	.001	.001	.001
23-24	.000+	.000+	.000+	.000	.000
24-25	.000	.000	.000		

Appendix B (continued)

Stable Age-Distribution for 21 Populations of Daphnia pulex
Exposed to Different Intensities of Continuous Gamma Radiation

(Each column in the body of the table refers to a single population.
The individual entries in each column are the proportion
of individuals of a given age, C_x , in that population.)

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:					
	64.16	67.20	67.53	69.66	70.70	75.93
0-1	.061	.044	.060	.074	.104	.024
1-2	.060	.044	.057	.071	.095	.026
2-3	.059	.046	.057	.068	.087	.026
3-4	.058	.047	.056	.065	.079	.029
4-5	.057	.048	.055	.063	.073	.032
5-6	.056	.049	.054	.060	.066	.034
6-7	.054	.050	.053	.057	.061	.037
7-8	.053	.051	.052	.055	.055	.040
8-9	.052	.052	.052	.053	.051	.044
9-10	.051	.053	.051	.050	.046	.048
10-11	.050	.054	.050	.048	.042	.052
11-12	.049	.055	.049	.046	.039	.056
12-13	.048	.056	.049	.044	.035	.059
13-14	.047	.057	.048	.042	.032	.064
14-15	.047	.059	.046	.041	.029	.070
15-16	.046	.056	.043	.039	.026	.076
16-17	.045	.056	.042	.037	.024	.082
17-18	.044	.054	.041	.032	.022	.077
18-19	.035	.038	.034	.026	.017	.068
19-20	.016	.023	.028	.016	.010	.032
20-21	.011	.005	.015	.010	.000+	.020
21-22	.002	.003	.005	.002	.000+	.003
22-23	.000	.001	.003	.000	.000	.000
23-24		.000	.000			

Appendix C

Age-Specific Survival Rates, l_x , for 21 Populations
of Daphnia pulex Exposed to Different Intensities
of Continuous Gamma Radiation

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:				
	0 (control no. 1)	0 (control no. 2)	0 (control no. 3)	22.80	24.03
0	1.00	1.00	1.00	1.00	1.00
1	1.00	1.00	0.98	0.98	1.00
2	0.98	1.00	0.96	0.98	1.00
3	0.98	1.00	0.96	0.98	1.00
4	0.98	1.00	0.96	0.98	1.00
5	0.98	1.00	0.96	0.96	1.00
6	0.98	1.00	0.96	0.98	1.00
7	0.98	0.98	0.96	0.98	1.00
8	0.98	0.98	0.96	0.98	1.00
9	0.98	0.98	0.96	0.98	1.00
10	0.98	0.98	0.96	0.98	1.00
11	0.98	0.98	0.96	0.96	0.98
12	0.96	0.98	0.96	0.96	0.96
13	0.96	0.98	0.96	0.96	0.96
14	0.96	0.98	0.96	0.96	0.96
15	0.96	0.98	0.96	0.96	0.92
16	0.96	0.98	0.96	0.94	0.92
17	0.96	0.98	0.96	0.94	0.90
18	0.92	0.98	0.94	0.94	0.88
19	0.90	0.98	0.94	0.94	0.81
20	0.90	0.98	0.92	0.90	0.81
21	0.84	0.98	0.88	0.88	0.79
22	0.82	0.91	0.84	0.82	0.79
23	0.80	0.81	0.68	0.76	0.67
24	0.69	0.58	0.56	0.73	0.58
25	0.57	0.49	0.48	0.69	0.48
26	0.51	0.35	0.40	0.63	0.38
27	0.35	0.28	0.36	0.59	0.27
28	0.25	0.19	0.24	0.53	0.25
29	0.20	0.14	0.10	0.39	0.23
30	0.14	0.14	0.10	0.37	0.21
31	0.08	0.14	0.06	0.31	0.17
32	0.08	0.07	0.00	0.27	0.13
33	0.06	0.05		0.20	0.08
34	0.02	0.05		0.10	0.02
35	0.00	0.00		0.00	0.00

Appendix C (continued)

Age-Specific Survival Rates, l_x , for 21 Populations
of Daphnia pulex Exposed to Different Intensities
of Continuous Gamma Radiation

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:				
	25.50	37.06	38.30	38.33	45.26
0	1.00	1.00	1.00	1.00	1.00
1	1.00	0.98	0.98	1.00	0.98
2	0.96	0.98	0.98	1.00	0.98
3	0.96	0.98	0.96	1.00	0.98
4	0.96	0.98	0.96	1.00	0.98
5	0.96	0.98	0.96	1.00	0.98
6	0.96	0.98	0.96	1.00	0.98
7	0.96	0.98	0.96	1.00	0.98
8	0.96	0.98	0.96	1.00	0.98
9	0.96	0.98	0.96	1.00	0.98
10	0.96	0.98	0.96	1.00	0.98
11	0.96	0.96	0.96	1.00	0.96
12	0.96	0.96	0.96	1.00	0.94
13	0.94	0.96	0.96	0.98	0.94
14	0.94	0.96	0.96	0.98	0.94
15	0.94	0.96	0.96	0.98	0.94
16	0.94	0.94	0.96	0.96	0.94
17	0.92	0.94	0.96	0.96	0.94
18	0.92	0.88	0.90	0.96	0.89
19	0.92	0.84	0.88	0.92	0.78
20	0.90	0.76	0.78	0.88	0.76
21	0.80	0.64	0.56	0.76	0.35
22	0.65	0.44	0.36	0.59	0.22
23	0.61	0.34	0.20	0.37	0.02
24	0.55	0.18	0.10	0.22	0.00
25	0.43	0.10	0.04	0.18	
26	0.39	0.00	0.00	0.06	
27	0.31			0.00	
28	0.20				
29	0.18				
30	0.16				
31	0.08				
32	0.06				
33	0.04				
34	0.00				

Appendix C (continued)

Age-Specific Survival Rates, l_x , for 21 Populations
of Daphnia pulex Exposed to Different Intensities
of Continuous Gamma Radiation

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:				
	47.94	48.46	52.23	53.73	54.13
0	1.00	1.00	1.00	1.00	1.00
1	0.98	1.00	1.00	1.00	0.98
2	0.98	0.98	0.98	1.00	0.96
3	0.98	0.98	0.98	1.00	0.96
4	0.98	0.98	0.98	1.00	0.96
5	0.98	0.98	0.98	1.00	0.96
6	0.98	0.96	0.98	1.00	0.96
7	0.98	0.96	0.98	1.00	0.96
8	0.98	0.96	0.98	1.00	0.96
9	0.98	0.96	0.96	0.98	0.96
10	0.98	0.96	0.96	0.98	0.96
11	0.98	0.96	0.94	0.98	0.96
12	0.98	0.96	0.94	0.98	0.96
13	0.98	0.96	0.94	0.98	0.94
14	0.98	0.96	0.94	0.98	0.94
15	0.98	0.96	0.94	0.98	0.94
16	0.96	0.96	0.94	0.98	0.94
17	0.96	0.96	0.94	0.94	0.92
18	0.96	0.94	0.72	0.88	0.82
19	0.88	0.82	0.51	0.70	0.67
20	0.70	0.54	0.31	0.50	0.31
21	0.48	0.16	0.16	0.16	0.14
22	0.24	0.14	0.07	0.06	0.06
23	0.04	0.04	0.01	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00

Appendix C (continued)

Age-Specific Survival Rates, l_x , for 21 Populations
of Daphnia pulex Exposed to Different Intensities
of Continuous Gamma Radiation

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:					
	64.16	67.20	67.53	69.66	70.70	75.93
0	1.00	1.00	1.00	1.00	1.00	1.00
1	1.00	1.00	0.98	1.00	1.00	0.98
2	1.00	1.00	0.98	1.00	1.00	0.96
3	1.00	1.00	0.98	1.00	1.00	0.96
4	1.00	1.00	0.98	1.00	1.00	0.96
5	1.00	1.00	0.98	1.00	1.00	0.96
6	0.98	1.00	0.98	1.00	1.00	0.96
7	0.98	1.00	0.98	1.00	1.00	0.96
8	0.98	1.00	0.98	1.00	1.00	0.96
9	0.98	1.00	0.98	1.00	1.00	0.96
10	0.96	1.00	0.98	1.00	1.00	0.96
11	0.96	1.00	0.98	1.00	1.00	0.96
12	0.96	1.00	0.98	1.00	1.00	0.94
13	0.96	1.00	0.98	1.00	1.00	0.94
14	0.96	1.00	0.96	1.00	0.98	0.94
15	0.96	0.94	0.92	1.00	0.98	0.94
16	0.96	0.92	0.90	1.00	0.98	0.94
17	0.96	0.86	0.90	0.90	0.98	0.81
18	0.78	0.60	0.76	0.76	0.84	0.67
19	0.36	0.36	0.64	0.48	0.51	0.29
20	0.26	0.08	0.34	0.32	0.24	0.17
21	0.04	0.04	0.12	0.06	0.06	0.02
22	0.00	0.02	0.06	0.00	0.00	0.00
23		0.00	0.00			

Appendix D

Age-Specific Fertility Rates, m_x ,
for 21 Populations of Daphnia pulex Exposed
to Different Intensities of Continuous Gamma Radiation

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:				
	0 (control no. 1)	0 (control no. 2)	0 (control no. 3)	22.80	24.03
4.5	0.020	0.000	0.040	0.063	0.063
5.5	2.180	1.674	1.680	1.520	1.896
6.5	0.560	0.965	0.940	0.667	0.958
7.5	3.380	2.643	2.160	2.271	2.771
8.5	0.800	2.071	1.460	1.479	1.208
9.5	2.400	2.000	1.920	1.958	2.583
10.5	1.660	3.095	2.460	1.292	1.167
11.5	1.414	1.952	1.160	1.916	2.215
12.5	1.653	2.881	2.540	1.915	1.761
13.5	0.265	0.975	0.340	0.489	1.848
14.5	0.083	2.762	0.420	1.043	2.333
15.5	0.000	0.071	0.080	0.000	1.023
16.5	0.021	0.095	0.240	0.022	0.713
17.5	0.083	0.000	0.000	0.000	0.094
18.5	0.237	0.024	0.260		0.025
19.5	0.022	0.000	0.020		0.000
20.5	0.135	0.119	0.240		
21.5	0.071	0.000	0.040		
22.5	0.104	0.027	0.220		
23.5	0.026	0.033	0.000		
24.5	0.219	0.135	0.269		
25.5	0.000	0.000	0.182		
26.5	0.045	0.148	0.000		
27.5	0.000	0.000	0.067		
28.5	0.087	0.143	0.118		
29.5	0.118	0.167	0.000		
30.5	0.000	0.000	0.250		
31.5			0.000		

Appendix D (continued)

Age Specific Fertility Rates, m_x ,
for 21 Populations of Daphnia pulex Exposed
to Different Intensities of Continuous Gamma Radiation

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:				
	25.50	37.06	38.30	38.33	45.26
4.5	0.000	0.061	0.042	0.061	0.111
5.5	1.894	1.898	1.375	1.837	1.867
6.5	0.830	0.959	1.292	0.388	0.733
7.5	2.979	1.612	1.271	1.959	1.067
8.5	0.340	0.959	0.958	0.388	0.244
9.5	0.362	0.367	0.104	0.408	0.067
10.5	0.021	0.289	0.271	0.245	0.135
11.5	0.064	0.313	0.146	0.286	0.138
12.5	0.025	0.708	0.313	1.010	0.023
13.5	0.044	0.292	0.104	0.188	0.140
14.5	0.022	0.896	0.729	0.813	0.047
15.5	0.044	0.105	0.000	0.126	0.070
16.5	0.044	0.021		0.021	0.000
17.5	0.000	0.000		0.000	
18.5	0.067				
19.5	0.000				

Appendix D (continued)

Age-Specific Fertility Rates, m_x ,
for 21 Populations of Daphnia pulex Exposed
to Different Intensities of Continuous Gamma Radiation

Age, x, in days	Population Exposed to Dose Rate (in Roentgens per Hour) of:				
	47.94	48.46	52.23	53.73	54.13
4.5	0.102	0.000	0.000	0.000	0.021
5.5	1.347	1.258	1.510	1.060	1.021
6.5	0.490	0.188	0.367	0.360	0.234
7.5	1.122	0.917	0.367	0.560	0.404
8.5	0.224	0.229	0.041	0.120	0.106
9.5	0.102	0.042	0.042	0.080	0.085
10.5	0.122	0.000	0.000	0.000	0.021
11.5	0.020				0.000
12.5	0.020				
13.5	0.000				
14.5	0.020				
15.5	0.000				

Appendix D (continued)

Age-Specific Fertility Rates, m_x ,
for 21 Populations of Daphnia pulex Exposed
to Different Intensities of Continuous Gamma Radiation

Age, x, in days.	Population Exposed to Dose Rate (in Roentgens per Hour) of:					
	64.16	67.20	67.53	69.66	70.70	75.93
4.5	0.040	0.000	0.000	0.000	0.082	0.022
5.5	0.545	0.520	0.673	0.720	1.040	0.174
6.5	0.449	0.160	0.265	0.400	0.286	0.196
7.5	0.082	0.180	0.163	0.180	0.286	0.196
8.5	0.000	0.040	0.020	0.000	0.020	0.022
9.5		0.000	0.000		0.000	0.000