



MORTALITY DYNAMICS OF DENSITY IN THE MEDITERRANEAN FRUIT FLY

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Abstract—The effects on medfly age-specific mortality of three types of densities—initial, current, and cumulative—were examined using sex-specific data from two sets of studies: (1) previous research on mortality patterns in 1.2 million individuals maintained in 167 different cages (1992 *Science* 258, 457) and ii) density experiments using a total of 210,000 individuals contained in 49 cages and maintained at one of three initial densities—2500, 5000 and 10,000 flies/cage. A central death rate was computed for each of the 216 cages at specified numerical levels (e.g., 5000, 4000, 1000, 500, 100, and so forth), which was distributed over a range of ages. This yielded a series of mortality schedules at “equivalent current densities.” Two main results are reported. First, the leveling off and decline in mortality at the most advanced ages as observed in the original study of 1.2 million medflies cannot be explained as an artifact of declining current densities at older ages. Second, increased initial density heightened the mortality level at each age but had essentially no effect on mortality pattern. The overall methodology and many of the results are believed to be general and thus both logistical and conceptual implications for gerontology and population biology are discussed.

Key Words: Mediterranean fruit fly, crowding, stress, sex mortality differentials, insect mortality

INTRODUCTION

MOST ANIMALS are widely spaced in nature due to behavioral patterns evolved to maintain territories (Tanner, 1966). Thus, when individuals are cohoused with conspecifics as is often the case in biological studies, stress is increased because behaviors associated with territoriality must be modified (Ranter and Boice, 1975; Price, 1984). This heightened stress, combined with an increase in the incidence of physical damage due to fighting and accidents in animals confined in close quarters, usually causes a

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decrease in longevity. The effects of crowding on longevity and physical injury is well documented in many insects including *Drosophila* (Pearl and Parker, 1922; Pearl *et al.*, 1927; Prout and McChesney, 1985; Graves and Mueller, 1993; Mueller *et al.*, 1993), the milkweed bug, *Oncopeltus fasciatus* (Dingle, 1968), the cotton stainer, *Dysdercus fasciatus* (Dingle, 1968), the corn earworm, *Heliothis zea* (Jones *et al.*, 1975), the dermestid beetle, *Trogoderma creutz* (Davis, 1945), the honey bee, *Apis mellifera* (Smaragdova, 1930), and the house fly, *Musca domestica* (Barber and Starnes, 1949; Patterson, 1957; Rockstein, 1957; Rockstein and Lieberman, 1959; Ragland and Sohal, 1973, 1975; Rockstein *et al.*, 1981; Finch, 1990). However, the effects of density on the underlying demographic determinant of the longevity differences—age-specific mortality—are virtually unknown. For example, it is not known whether crowding reduces life expectancy in cohorts by altering the overall pattern of the mortality schedule, by increasing the slope of the mortality schedule over particular age groups, or by increasing the level of mortality at each age. Nor is it known whether different levels of density have qualitatively different effects on mortality patterns of subgroups such as males vs. females or short- vs. long-lived strains.

Understanding the mortality dynamics of density—how different numbers of animals per cage affects the levels and patterns of cohort mortality—is important for several reasons. The first reason concerns the use of parameters derived from mortality models for inter- and intraspecific comparisons. The books by Comfort (1979), Finch (1990), and Gavrilov and Gavrilova (1991) all contain life-table information gathered from the literature on scores of species and include estimates of parameters derived from the Gompertz or the Gompertz-Makeham mortality models (Gompertz, 1825; Makeham, 1867) such as the initial mortality rate, the exponential coefficient (age-dependent component), and the Makeham constant (age-independent mortality). Because the majority of life-tables cited in these books were constructed from data on confined cohorts, the use of the parameters for comparing the mortality properties of groups, species, or strains is questionable if initial density affects any aspect of the mortality schedule. The same concept is relevant to selection experiments designed to isolate “density tolerant” strains of *Drosophila* (Mueller *et al.*, 1993). It is not known, for example, whether longer life spans in strains that have been selected to live longer under crowded conditions are due to a delay in the onset of senescence, to a decrease in the exponential rate of aging (age-dependent component), or to a uniform reduction in mortality at each age (age-independent component).

A second reason that understanding the effects of density on age-specific mortality is important concerns gender differences in longevity. If the stress and wear and tear caused by crowding affects the mortality rate in one sex more than the other, then the outcome of many experiments on sex-specific mortality differences (e.g., Hamilton, 1948) may be an artifact of density effects. It is conceivable that life expectancy in some species maintained under high densities could favor one sex but favor the opposite sex if maintained under low densities; thus, which sex lives longer is conditional on environmental parameters.

A third reason that understanding the effects of density on age-specific mortality is important concerns the findings in previous studies on fruit flies (Carey *et al.*, 1992; Curtsinger *et al.*, 1992) that mortality rates level off and decline at the most advanced ages. This interpretation was challenged by others (Kowald and Kirkwood, 1993; Nusbaum *et al.*, 1993; Robine and Ritchie, 1993; also see Carey *et al.*, 1993a,b), who argued

that the leveling off and decline was likely an artifact of density effects—mortality decreased due to declining densities with age. The interpretation by Carey *et al.* (1992) and Curtsinger *et al.* (1992) is wrong if this explanation can account for the deceleration of mortality at older ages. However, if density effects do not alter the overall pattern of mortality at older ages, then the interpretation stands and the leveling off and decline of mortality at advanced ages will have to be seriously addressed by gerontologists, evolutionary biologists, entomologists, demographers, and others.

Because information on the effects of density on mortality is almost nonexistent and because the mortality schedule is fundamental to life-table studies on aging and senescence (Manton and Stallard, 1984; Gavrilov and Gavrilova, 1991), the broad objective of this article is to report the findings on the effects of different levels of crowding on age-specific mortality in the Mediterranean fruit fly, *Ceratitis capitata*, using the large-scale experimental system described in Carey *et al.* (1992). Our specific objective in the study was to test the hypothesis that the overall pattern of mortality in the medfly and particularly the leveling off and decline at older ages is independent of initial, current, and cumulative densities. Although we use the medfly as a model, we believe that our findings have implications that pertain to any life table and aging study of confined organisms.

MATERIALS AND METHODS

Types of density effects

Density effects can be separated into three types (Fig. 1): (1) initial density—the starting number of individuals in each cohort; (2) cumulative density—the cumulative number of surviving flies in a cage up to day x ; and (3) current density—the average number of flies alive at age x . Initial density is often used as a proxy for the other two types of densities. However, initial density is a constant in that it does not involve the concept of fly days or duration of exposure. Cumulative density is the sum total of fly days, and current density is the total number of flies in a cage at a specified time. Initial density is constant, and cumulative density increases with age so neither factor can account directly for a decline in mortality at older ages. The three types of densities are not independent.

Experimental details

Two sets of data were used in the overall study, both of which were gathered at the Moscamed medfly mass rearing facility located in Metapa, Chiapas, Mexico (see Vargas, 1989, for technical details). The first set of data were those gathered for Experiment 3 described in Carey *et al.* (1992). Medflies of both sexes were maintained in mesh-covered 15 by 60 by 90 cm aluminum cages at 12:12 LD cycle, 24.0°C ($\pm 2^\circ$) and 65% RH ($\pm 9\%$). Adults were given water and a diet of sugar spread on a 50 cm² sheet of paper suspended from the top of each cage. Each day dead flies were removed, counted, and their sex was determined. Data for a total of over 1.2 million medflies was gathered from 167 cages containing an average of about 7200 flies each. Densities varied from the target density because of variability in the number of pupae technicians placed in a cage and in the proportion of pupae that successfully emerged and survived to the beginning of the first day. The technicians placed approximately the same volume of

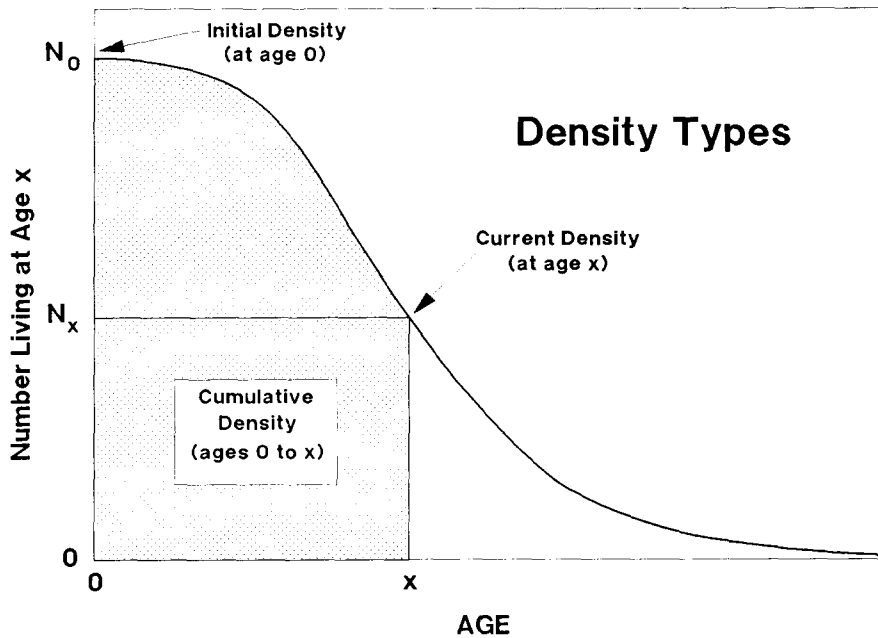


FIG. 1. Survival in a hypothetical cohort showing the three types of densities: (1) initial density, which is the number of individuals at age 0; (2) current density, which is the number of individuals alive at age x ; and (3) cumulative density, which is the total number of fly days from day 0 through age x .

pupae in each cage, but depending on the size of the pupae, two equal volumes can contain different numbers.

The second set of data was gathered using an experiment explicitly designed to measure density effects on medfly mortality. On the same day and using the same batch of pupae, one cage with about 10,000 flies, two cages with about 5000 flies, and four cages with about 2500 flies were set up. This procedure was repeated seven times for a total of 49 cages maintained under the same environmental conditions as in Experiments 3 described in Carey *et al.* (1992). Mortality data was obtained on a total of 214,735 flies or around 70,000 flies for each of the three treatments. Because no larval food (diet) was available in cohort cages, it was not possible for eggs laid by females to develop into second generation adults and, thus, contaminate the cohort with younger aged flies.

Life-table methods

Four life-table parameters were used in the data analysis (Chiang, 1984; Pressat, 1985): (1) age-specific mortality, q_x , defined as the fraction of flies alive at age x ; dying in the interval x to $x + 1$; (2) cohort survival, l_x , defined as the fraction of the original number of flies age 0 that survive to age x ; (3) expectation of life at age x , e_x , defined as the average number of days remaining to a fly age x ; and (4) central death rate, m_x , defined as the number of deaths occurring in a specified period of time and in a specific age-sex category divided by the population at risk (typically the midperiod population).

A measure of the geometric rate of change in mortality with age is given by the term $R_x = q_{x+1}/q_x$. If $R_x > 1$, then mortality is increasing at age x ; if $R_x = 1$, then mortality is unchanging at age x ; and if $R_x < 1$, then mortality is decreasing at age x . A measure of the relative survival of males and females in a cohort as the cohort ages is reflected in the sex survival ratio, $S_x = l_x^m/l_x^f$, where superscripts m and f denote male and female schedules, respectively. If $S_x > 1$, then male survival to age x is greater than female survival to this age; if $S_x = 1$, then male and female survival are equal to age x ; and if $S_x < 1$, then male survival is less than female survival to age x . The ratio of the number of males to the number of females at age 0 (initial numerical sex ratio) in most cages departed from a 1:1 ratio due to slight biases in size of pupae (female pupae are, on average, slightly larger than male pupae) and due to chance. Approximately 90% of the cages had male-to-female ratios ranging from 0.80 to 1.40 with an average male–female numerical ratio of 1.01 (SD ± 0.206).

Age-density models

Because density declines with age in life-table experiments where dead individuals are not replaced, the observed age trajectory of medfly mortality could be an artifact of the shifting balance between an age effect and a density effect. Let $m(x, N)$ be the death rate at age x in a cage with N flies. Then the two propositions underlying the hypothesis are: (1) for cages at the same current density, death rates increase with age, i.e., $\partial m(x, N)/\partial x > 0$. (2) For flies at the same age, death rates increase with density, i.e., $\partial m(x, N)/\partial N > 0$. Because density N declines with age, i.e., $dN(x)/dx < 0$, death rates can either increase or decrease with age:

$$\frac{dm(x, N(x))}{dx} = \frac{\partial m(x, N)}{\partial x} \Big|_{N=N(x)} + \frac{dN(x)}{dx} \frac{\partial m(x, N)}{\partial N} \Big|_{N=N(x)} \tag{1}$$

The first term in this expression can be interpreted as the age effect and the second term as the density effect. If at younger ages the age effect outweighs the density effect but at older ages the density effect outweighs the age effect, mortality will rise and then fall.

To simultaneously control for density and differential mortality, we estimated, separately for each day, the coefficients $m^o, a(x), b(x)$, and $\delta(x)$ of the model:

$$\ln m_i(x) = \ln m^o(x) + a(x)\ln N_i(x) + b(x)\ln(H_i(x)/\bar{H}(x)) + I_i \ln \delta(x) + \epsilon_i(x) \tag{2}$$

where $m_i(x)$ is the central death rate at age x in cage i , $N_i(x)$ is the number of surviving flies in cage i at the start of day x , $H_i(x)$ is the cumulative hazard, \bar{H} is the cumulative hazard for all cages combined, I_i equals one for the cages in the density experiment and zero for the cages in the original 1.2 million medfly experiment, and ϵ_i is the error. The coefficient m^o is the estimated baseline death rate controlling for density and differential mortality. The central death rate is given by

$$m_i(x) = \frac{N_i(x) - N_i(x + 1)}{(N_i(x) + N_i(x + 1))/2} \tag{3}$$

The cumulative hazard can be calculated by $H_i(x) = -\ln[N_i(x)/N_i(0)]$. Note that the ratio $H_i(x)/\bar{H}(x)$ can be interpreted as the average relative risk of mortality in cage i up to age x : if death rates in cage i are z times the average for all cages, then $H_i/\bar{H} = z$. We estimated the coefficients using the least squares criterion in a multiple regression analysis. In some cages at some ages, especially advanced ages, there were no deaths. To avoid values of zero for $m_i(x)$ and to smooth the erratic death rates observed in cages with few survivors, we substituted for $m_i(x)$ the average death rate in the interval $x \pm k$ for all cages with no deaths or with fewer than 10 survivors, using the formula

$$m_i(x) = \frac{N_i(x - k) - N_i(x + k)}{\sum_{j=-k}^k (N_i(x + j) + N_i(x + j + 1))/2} \quad (4)$$

We used the smallest value of k such that there was at least one death in the interval and at least 10 fly days of exposure. In the regression analysis, we excluded cages with no surviving flies on day x .

We also estimated regression equations with the additional term $\theta(x)\ln[m_i(x - 1)/\hat{m}_i(x - 1)]$ added after the initial day to account for serial correlation between the estimation error on successive days: $\hat{m}_i(x - 1)$ is the value estimated by the regression equation for the previous day.

Daily age-specific death rates at specified densities were computed over a wide range of densities from one fly per cage to 5000 flies per cage for (1) the density experiment; and (2) the density experiment and the original 1.2 million medfly experiment combined. Values were estimated for successive 10-day intervals and were plotted at the midpoint of the relevant interval. Within each interval, death rates for a specified density were based on death rates in the cages that achieved this density on some day in the interval, the death rate used being the rate on the day the density was achieved. For the interval running from the start of day x to the start of day $x + 10$ and for density D , the death rate m was calculated by

$$m = \frac{\sum_{k=0}^9 I(N_i(x + k))[N_i(x + k) - N_i(x + k + 1)]}{\sum_{k=0}^9 I(N_i(x + k))[N_i(x + k) + N_i(x + k + 1)]} \quad (5)$$

where $I[N_i(x + k)] = 1$ if $N_i(x + k) \geq D$ and $N_i(x + k + 1) \leq D$ and $I[N_i(x + k)] = 0$ otherwise, where $N_i(x)$ is the number of surviving flies in cage i at the start of day x . Values of m were plotted only if there were at least 50 days of exposure at the specified density and age interval, i.e., the denominator of the formula has to exceed 50.

RESULTS

Density effects on survival and life expectancy

A demographic summary by sex for the three density treatments is presented in Table 1. Several aspects of this table merit comment. *First*, the two- to fourfold differences in number of flies per cage at the beginning of the experiments were reduced to around 1.5- to 2.5-fold differences by 20 days due to the higher mortality in the higher density cages. The relative differences in numbers due to different initial densities diminished with time and were virtually nonexistent by around 4 to 5 weeks. *Second*, the fraction of the original cohort surviving to 20 and 40 days was inversely related to initial number. For example, there was about fivefold greater survival of females to 40 days in cages starting with 2500 individuals relative to survival of females to this age in cages starting with 10,000 individuals. *Third*, the sex and density trends were also reflected in life expectancies at different ages. For example, life expectancies at eclosion (day 0) for both males and females were less in higher density cages than in lower density ones. Also, male life expectancy at eclosion was 16% greater in low-density cages than in high-density cages, whereas female life expectancy at eclosion was 23% greater in the

TABLE 1. SUMMARY OF RESULTS FOR MALES AND FEMALE MEDFLIES MAINTAINED IN MIXED CAGES AT THREE INITIAL DENSITIES—2,500, 5,000 and 10,000 FLIES PER CAGE

Parameter at age x	Males			Females		
	2,500	5,000	10,000	2,500	5,000	10,000
Number per cage ^a						
x = 0	1279 (292.9)	2568 (512.7)	5189 (826.3)	1253 (329.0)	2577 (490.1)	5068 (948.2)
20	560 (206.5)	997 (543.3)	1568 (815.5)	409 (151.9)	615 (113.0)	854 (201.6)
40	18 (13.3)	29 (28.2)	26 (29.1)	26 (15.7)	25 (13.6)	20 (12.4)
Survival to age x (l_x) ^b						
x = 20	0.4380 (0.110)	0.3882 (0.152)	0.3022 (0.123)	0.3263 (0.112)	0.2385 (0.080)	0.1685 (0.061)
40	0.0140 (0.009)	0.0113 (0.009)	0.0050 (0.005)	0.0206 (0.014)	0.0096 (0.006)	0.0039 (0.003)
Expectation of life (e_x) ^c						
x = 0	19.2 (1.92)	18.3 (2.67)	16.6 (2.14)	17.5 (2.31)	15.7 (1.42)	14.2 (1.01)
20	6.6 (0.95)	6.4 (1.36)	5.5 (0.88)	8.2 (1.25)	6.8 (1.10)	6.0 (0.66)
40	5.9 (2.56)	6.0 (1.65)	5.4 (1.76)	7.7 (2.46)	5.9 (1.22)	5.7 (1.47)

Values are per cage averages (SD) using 28 cages of approximately 2,500 flies, 14 cages of approximately 5,000 flies and 7 cages of approximately 10,000 flies.

^aNumber by sex; sum of numbers of both sexes gives average cage densities at specified ages.

^bFraction of the original cohort surviving to age x.

^cNumber of days remaining to the average individual alive at age x.

low- vs. high-density cages. These findings are similar to those of Dingle (1968) who reported that *Oncopeltus* and *Dysdercus* females experienced proportionately higher mortality than males at high vs. low densities. However, Rockstein *et al.* (1981) reported the opposite effect of density on male–female differences in life expectancy in *Musca domestica*. Anderson (1961) examined the effects of density on sex ratio in a wide range of species but reported little that was generalizable. *Fourth*, a male–female mortality crossover was evident at all three densities and is consistent with findings from analysis of male/female data from original life-table study on 1.2 million medflies (Carey *et al.*, 1992). For example, expectation of life was greater for males than for females at age 0 but less for males than for females at 40 days.

Effects of initial density on mortality

Comparisons of the average mortality ratios among the three crowding experiments are given in Table 2 for each sex. Relative differences between high- and medium-density cages were similar for males and females, averaging around 1.20-fold greater in the higher density cages. However, male mortality averaged only 1.10-fold higher in medium-density cages relative to low-density cages, whereas female mortality averaged 1.19-fold higher. In general, each doubling of initial density increased mortality for the first 40 days by 10 to 20% which, in turn, decreased life expectancy at age 0 by about 5 to 10%.

The smoothed age-specific mortality schedules for all density treatments for both sexes are presented in Fig. 2, which shows the uniformity of the three sex-specific mortality patterns corresponding to each treatment. In all treatments, male mortality increased monotonically to around day 20, abruptly leveled off from days 20 through 40, and decreased thereafter. Female mortality increased to around day 18, at which time it continued to increase at a slower rate for 2 more weeks. At around day 35,

TABLE 2. AVERAGE MORTALITY RATIO (SD)
BY SEX FOR THREE DENSITY EXPERIMENTS
FROM DAY 0 THROUGH 40

<i>Mortality ratio</i>	<i>Males</i>	<i>Females</i>
High-to-medium	1.20 (0.130)	1.19 (0.131)
Medium-to-low	1.10 (0.120)	1.19 (0.106)
High-to-low	1.32 (0.209)	1.42 (0.186)

High, medium, and low densities denote 10,000, 5,000, and 2,500 flies/cage, respectively. For example, the average male mortality at ages 0 through 40 days was 1.20-fold greater in the high density cages (10,000 flies/cage) than in the medium density cages (5,000 flies/cage).

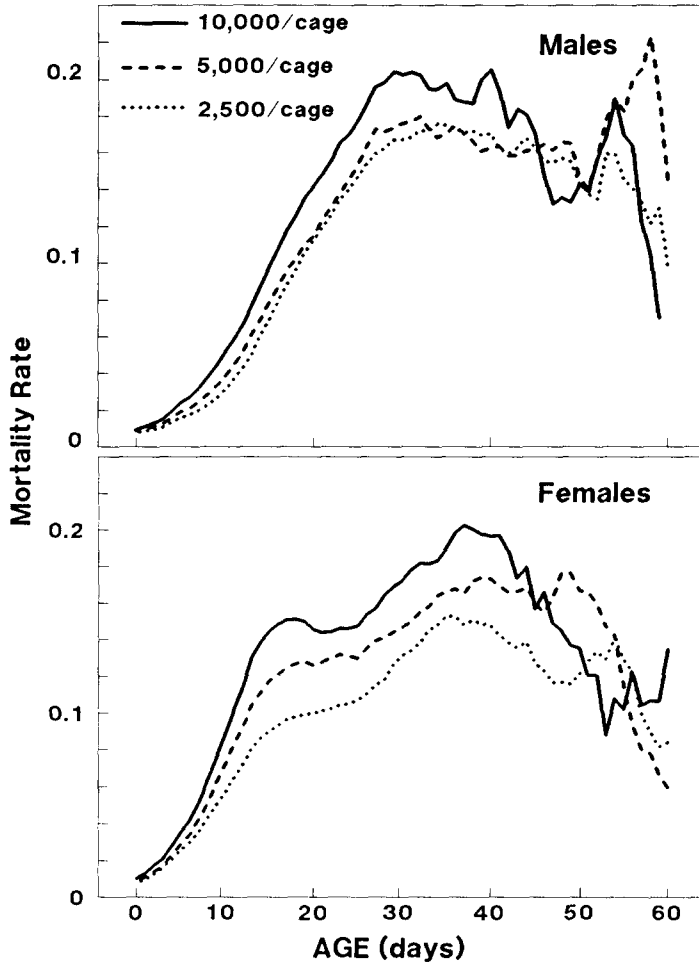


FIG. 2. Smoothed (7-day geometric mean) age-specific mortality (q_x) for males (top) and female (bottom) medflies at three different initial densities. Each curve is based on an initial number of approximately 35,000 individuals (each sex). Age-specific mortality is defined as the fraction of flies alive on day x that die in the interval x to $x + 1$.

female mortality rates began to decrease for all three density treatments. In short, mortality differences among the three treatments were quantitative and not qualitative; the shapes of all the schedules were similar to the mortality schedules observed in the 1.2 million medfly cohort (Carey *et al.*, 1992; Carey, 1993). The leveling off of mortality observed in this study was also observed in *Drosophila* by Curtsinger *et al.* (1992), Fukui *et al.* (1993), and Clark and Guadalupe (1993), and in bean beetles by Tatar *et al.* (1994) and Tatar and Carey (1994a,b). We also found similar patterns of rising, level, and then decreasing death rates in the life-table study by Krainacker (1986) and Krainacker *et al.* (1987) based on around 2000 medflies maintained in cages of 25 to 50 pair.

Effects of initial density on mortality patterns

The rate of change at each age for the sex-specific mortality curves are presented in Fig. 3. These plots reveal the following relationships between density and mortality: first, the geometric rate of change in mortality rates declines to unity for cohorts from all density treatments and for both sexes. This supports the hypothesis that slowing of mortality at older ages is independent of initial density. Second, the ages at which the rate of change in mortality reaches unity are similar among the three treatments (i.e., the three initial densities)—approximately 27 days for males and 20 days for females. That the convergence age is independent of the initial number suggests that the slowing of mortality at these older ages is *not* conditional on decreases in density. Third, the

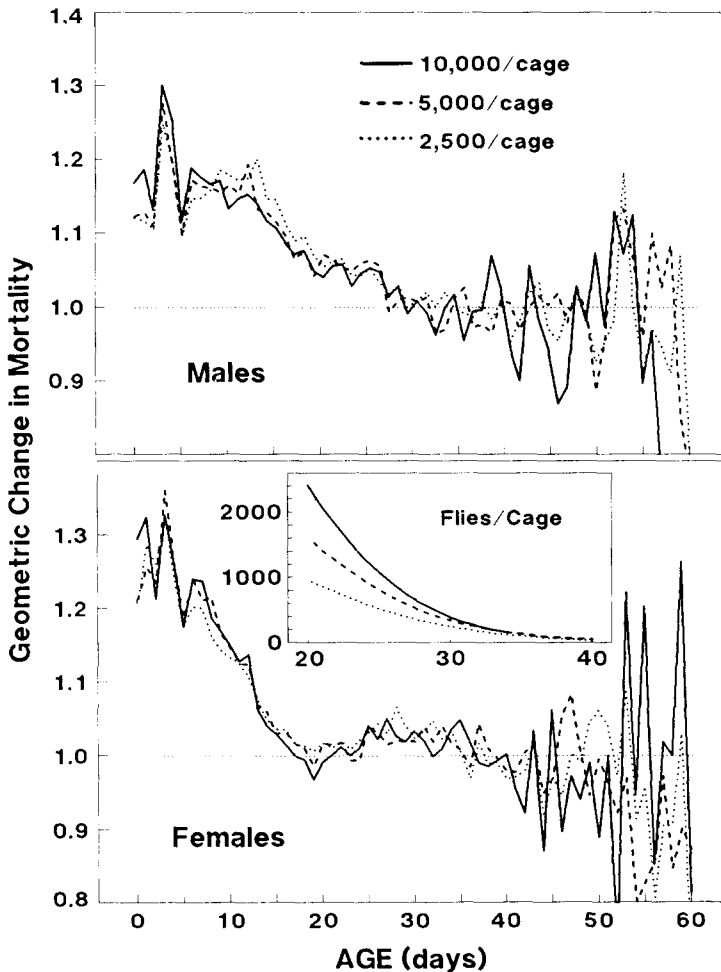


FIG. 3. Geometric rate of change in age-specific mortality (q_{x+1}/q_x) for males (top) and female (bottom) medflies at three different initial densities. The inset shows the average total number of individuals in each cage from days 20 through 40 for the three treatments.

average number of individuals within a cage differs by several-fold (lower inset in Fig. 3) at the respective ages of the convergence of mortality rate of change to 1.0. For example, at 20 days when the rate of change in female mortality converged to 1.0, the densities in the three treatments ranged from 2500 to 1000 flies/cage. This implies that no "threshold density" exists such as a critical per fly spacing requirement (Carpenter, 1958; Connolly, 1968; Luckinbill and Clare, 1986).

The relationship shown in Fig. 3 also serves as a test of the hypothesis that demographic heterogeneity accounts for the leveling off, as was suggested by Kowald and Kirkwood (1993), Hughes and Charlesworth (1994), and Brooks *et al.* (1994). This hypothesis assumes that the cohort consists of subcohorts with different levels of frailty but that all subcohorts exhibit Gompertz mortality rates. Therefore, it is argued, as the cohort ages it becomes more selected, because the subgroups with higher death rates die out leaving the more robust subgroups with lower death rates (see Vaupel *et al.*, 1979; Kowald and Kirkwood, 1993; Vaupel and Carey, 1993). Thus, it is suggested that the differential mortality among subgroups that exhibit Gompertz mortality patterns creates the non-Gompertzian pattern of leveling off in the whole cohort. A test of this hypothesis became possible when we observed that changes in initial densities scaled mortality uniformly across all ages. The concept for testing the hypothesis is this: if mortality is increased uniformly across all subgroups then, if heterogeneity accounts for the departure of the mortality pattern from the Gompertz (exponential), leveling off should occur at younger ages when total mortality is high than when total mortality is low. This shift in timing would occur because survivorship decreases more rapidly at high levels of mortality than at low ones and, thus, individuals in the most robust subgroups should be the predominant mortality type at an earlier age. However, this was not the case with the medflies, as is evident in Figs. 2 and 3; the timing of the deceleration was independent of the level of mortality that resulted from different initial densities. Therefore, we conclude that heterogeneity does not explain the leveling off of mortality in the medfly cohorts. This finding does not rule out the existence of demographic selection. Rather, it suggests that the effects of changes at the level of the individual (e.g., reproductive physiology) supercede the effects of demographic heterogeneity and selection; in short, the leveling off of mortality is not an artifact of changes in cohort composition.

Correlation of density and mortality

There was a high correlation between initial density and cumulative density (Fig. 4), which suggests that initial density can be used as a proxy for cumulative density. The correlation coefficients for both cumulative density and current density vs. mortality at each age for days 0 through 40 days are plotted in Fig. 5. This figure shows (1) a moderately high correlation for females between mortality and density (both cumulative and current) for 0 through 14 days but a low correlation between mortality and the two density measures for males at all ages; and (2) that the correlation coefficients for mortality and current density are virtually identical with those for cumulative density at young ages (< 10 days) but diverge at older ages. This overall pattern is due to the direct age dependence of the two types of densities.

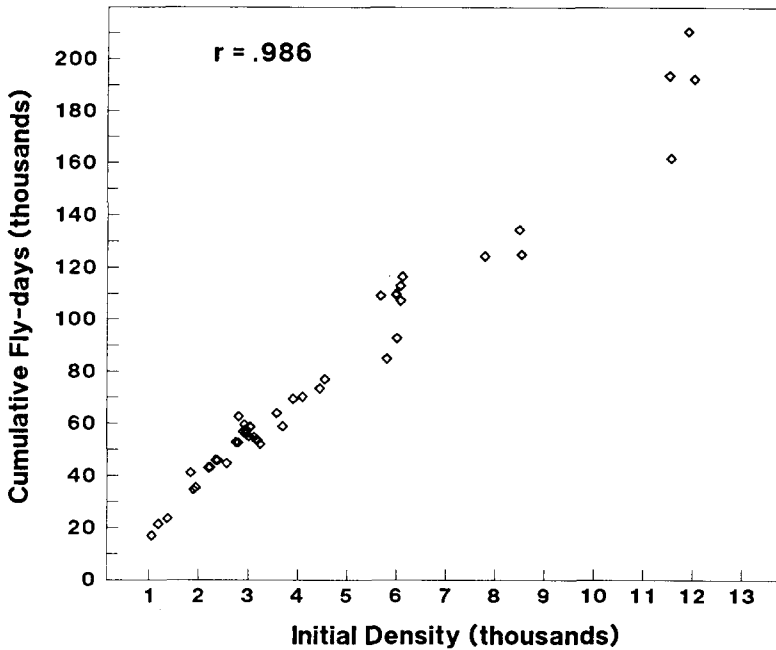


FIG. 4. Relationship between initial density and cumulative density (fly days summed through day 40) for medfly density experiments.

Density effects on sex survival ratios

We examined two sets of age-specific survival ratios for the two sexes in the density studies. The first was the ratio of the survival schedule computed for each sex in the lowest density experiment (2500 flies/cage) to the survival schedule of the respective sex in the highest density experiment (10,000 flies/cage), the results of which are given in Fig. 6. These results show that the long-term effects of density on female survival is much greater than the effects on males and continues to increase for nearly 80 days. By 40 days, the lower density cages contain an average of sixfold more of their initial number of female flies than the higher density cages. In contrast, at this same time the lower density cages contain an average of threefold more of their initial number of male flies than the higher density cages. This reinforces the findings reported earlier that high initial density has a greater effect on female mortality than on male mortality.

The second set of survival ratios that we examined were the male:female survival ratios for each of the three densities, the results of which are shown in Fig. 7. This figure shows two important patterns. First, the increasing male:female survival ratio to 20 days followed by the decreasing survival ratio for at least the next 10 days in all cohorts reveals the male:female mortality crossover. The relative abundance of male medflies could not increase and decrease without a mortality crossover—male mortality lower at young ages and female mortality lower at older ages. Second, the higher density cages amplify the relative differences favoring males. Because females die off at a much faster rate than do males at the young ages, the relative advantage of females after the mortality crossover does not offset the sex bias created by the differentials

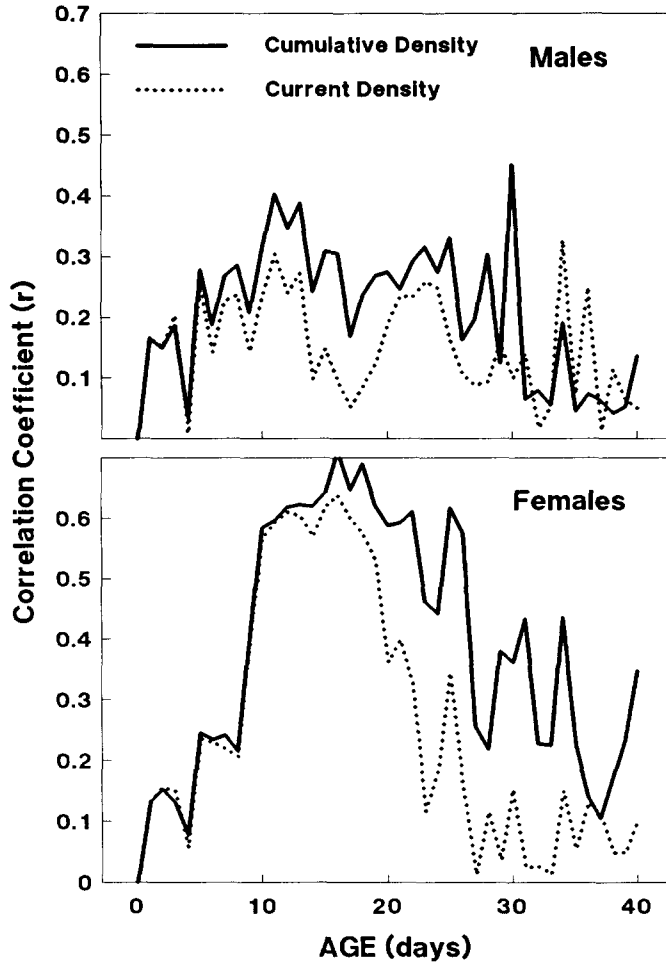


FIG. 5. Correlation coefficients for cumulative and current density vs. mortality at each age for male (top) and female (bottom) medflies. Results are based on seven cages with an average density of about 10,000 medflies, 14 cages with an average density of about 5000 medflies, and 28 cages with an average density of about 2500 medflies.

during the first 3 weeks. Therefore, the survival schedules do not cross over until nearly 60 days. In short, the quantitative differences in sex-specific mortality accounted for the qualitative difference in sex bias at the older ages; for the medfly, sex bias at older ages is partly attributable to the effects of crowding.

Equivalent current densities

The underlying concept for the analysis of “equivalent current densities” is that each cohort will progressively decline through all numerical levels ranging from the initial number to zero (extinction) as its members die off. Because of differences in starting numbers and of mortality among cages, there will exist variation among cages in the day

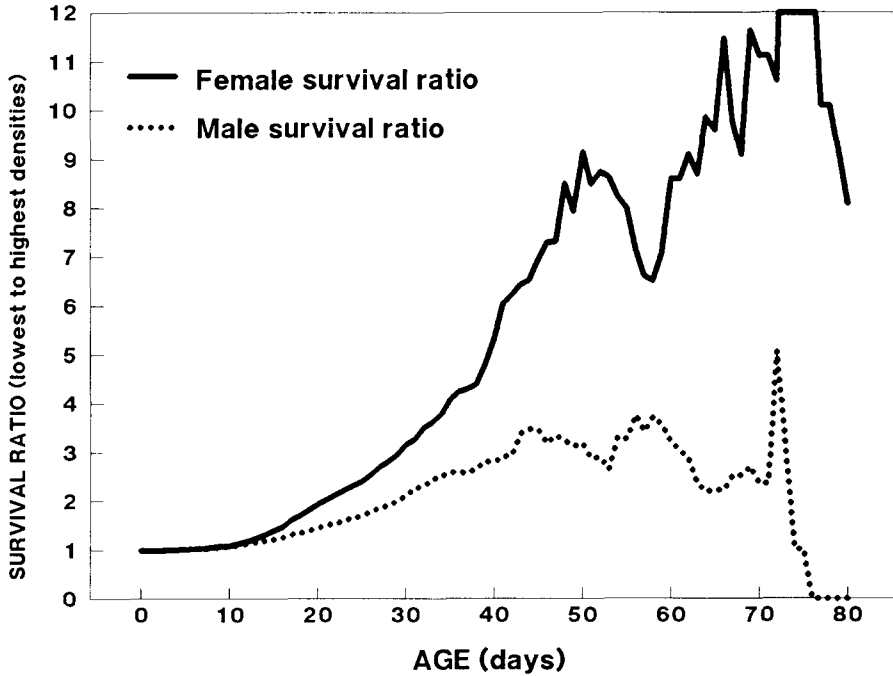


FIG. 6. Ratio of the sex-specific survival schedules for the low (2500 flies/cage) vs. the high (10,000 flies/cage) density experiments. For example, there were ninefold more female survivors in the low-density trials than in the high-density trials at day 50, and threefold more male survivors in the low- vs. high-density trials on day 40.

at which a particular numerical level is attained. A central death rate can be computed for each of the 216 cages (cohorts) at specified numerical levels (eg. 5000, 4000, 1000, 500, 100, and so forth), which will, in turn, be distributed over a range of age classes. This will yield a series of mortality schedules at "equivalent current densities" as shown in Figs. 8A,B. These schedules can then be pieced together to produce composite schedules spanning all age classes, as shown in Figs. 9A,B. Holding density constant, death rates tend to rise at younger ages, stay approximately level at middle ages, and fall at older ages. For example, the central death rate in cages with one to five flies at 50 days was 0.3 or greater whereas the central death rate in cages with one to five flies 30 to 60 days later was 0.1 or less. Thus, cages with equivalent current densities exhibited different death rates at different ages. At most ages death rates are roughly the same at different densities, with a tendency for death rates to be lower at higher densities.

The data underlying Figs. 8A,B and 9A,B permit 151 paired comparisons of cohorts of the same age but different current densities. For example, in the age interval from 10 through 19 days, death rates were available for seven densities—400, 500, 1000, 2000, 3000, 4000, and 5000 flies per cage. This permitted paired comparisons of whether mortality rates were higher or lower at the higher densities. For all age intervals combined, a total of 151 paired comparisons could be made. In 74% of the cages, death rates were lower in the cage with the higher density, a highly significant result ($p < 10^{-8}$).

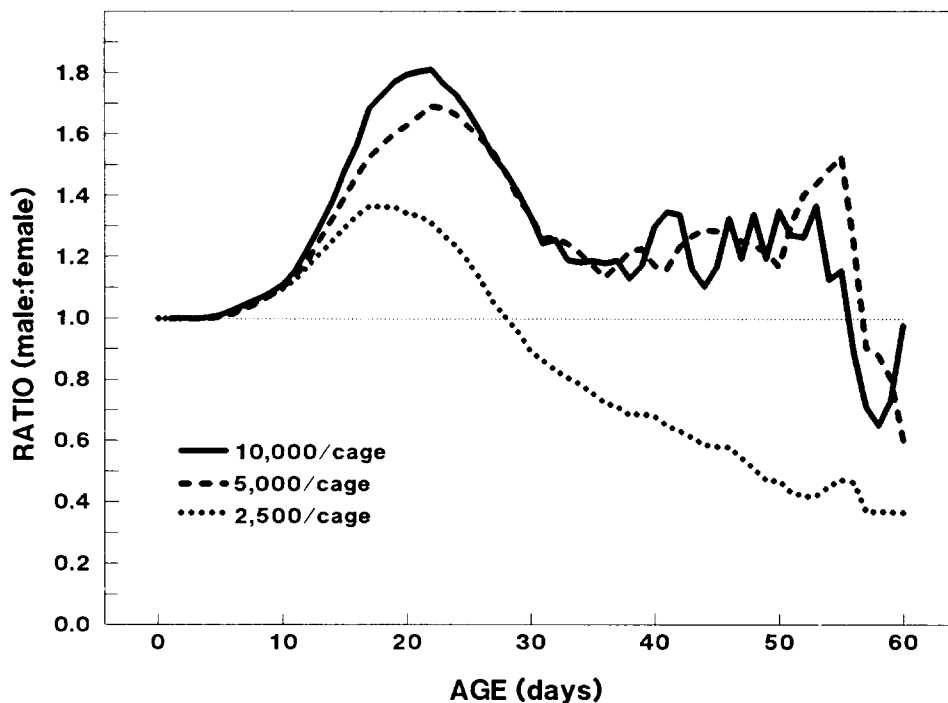
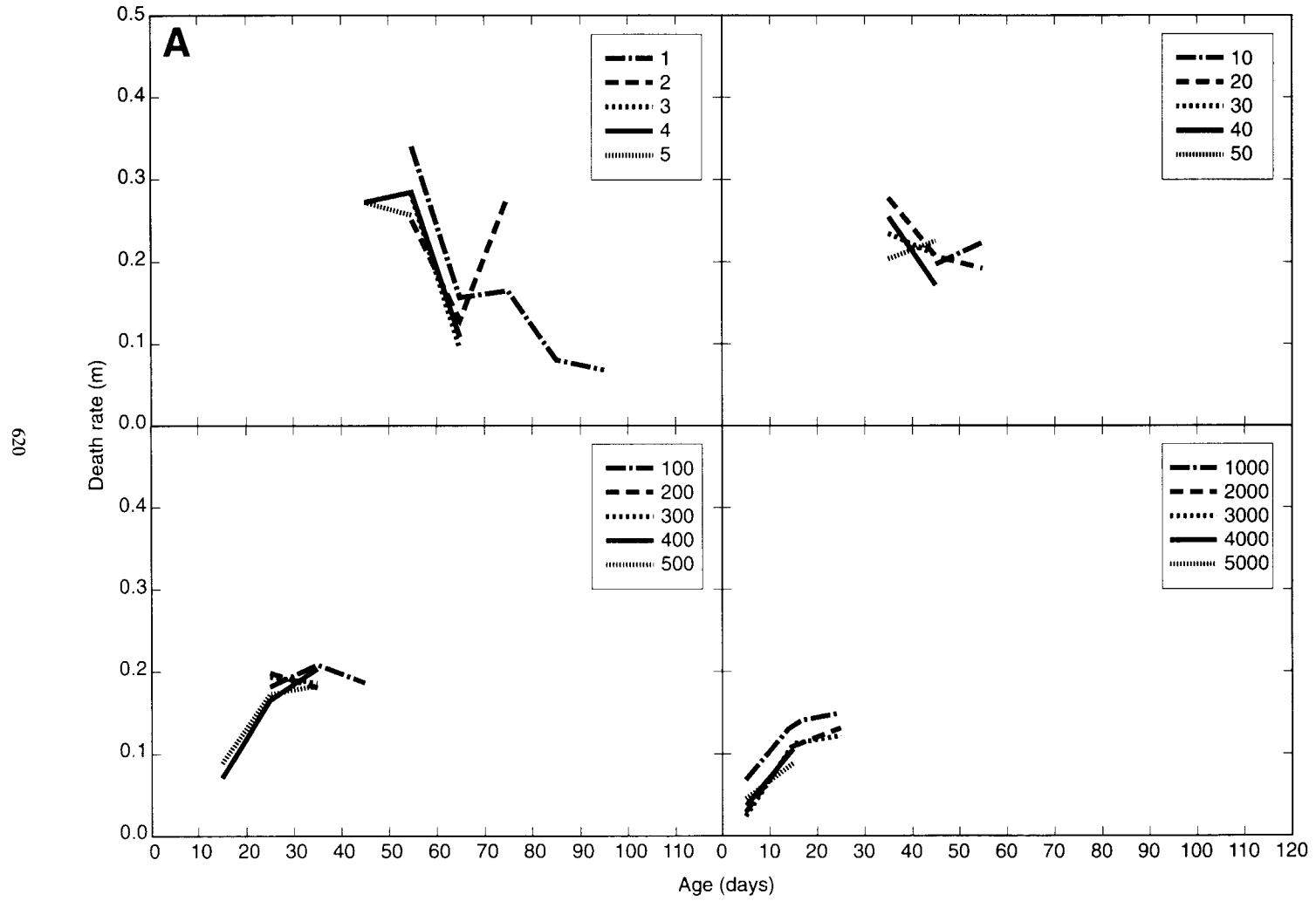


FIG. 7. Ratio of the male medfly survival schedule to the female medfly survival schedule for each of the three initial cage densities.

The data from the 49 cages in the density experiment was combined with data from the 167 cages in the original 1.2 million medfly experiment (Table 3). The results are similarly inconsistent with proposition A (for cages at the same current density, death rates increase with age) and with proposition B (for flies at the same age, death rates increase with density).

The unexpected negative density effect observed in the experiments may be explained as due to two possibilities. The first possibility concerns cohort heterogeneity. Some cages may have contained disproportionate number of robust flies; environmental conditions in some cages may have been especially salubrious. "Good" cages would tend to experience relatively low mortality and, thus, reach a particular density at a later age than bad cages. Consistent with this hypothesis, mortality in the 10 days following day x is positively correlated with mortality up to day x , at younger ages. However, at the middle and older ages when the decline in death rates occurs, the correlation is weak and insignificant (Table 4).

The second possibility is that there exists a complex relationship in cohort numbers (current densities) and the effects of the autocorrelation between earlier and later mortality. It may be that high initial density raised mortality so much at young ages that the high initial density cases turn into low-current density cases at later ages. In other words, because of the autocorrelation between earlier and later mortality, current density starts becoming inversely related to mortality.



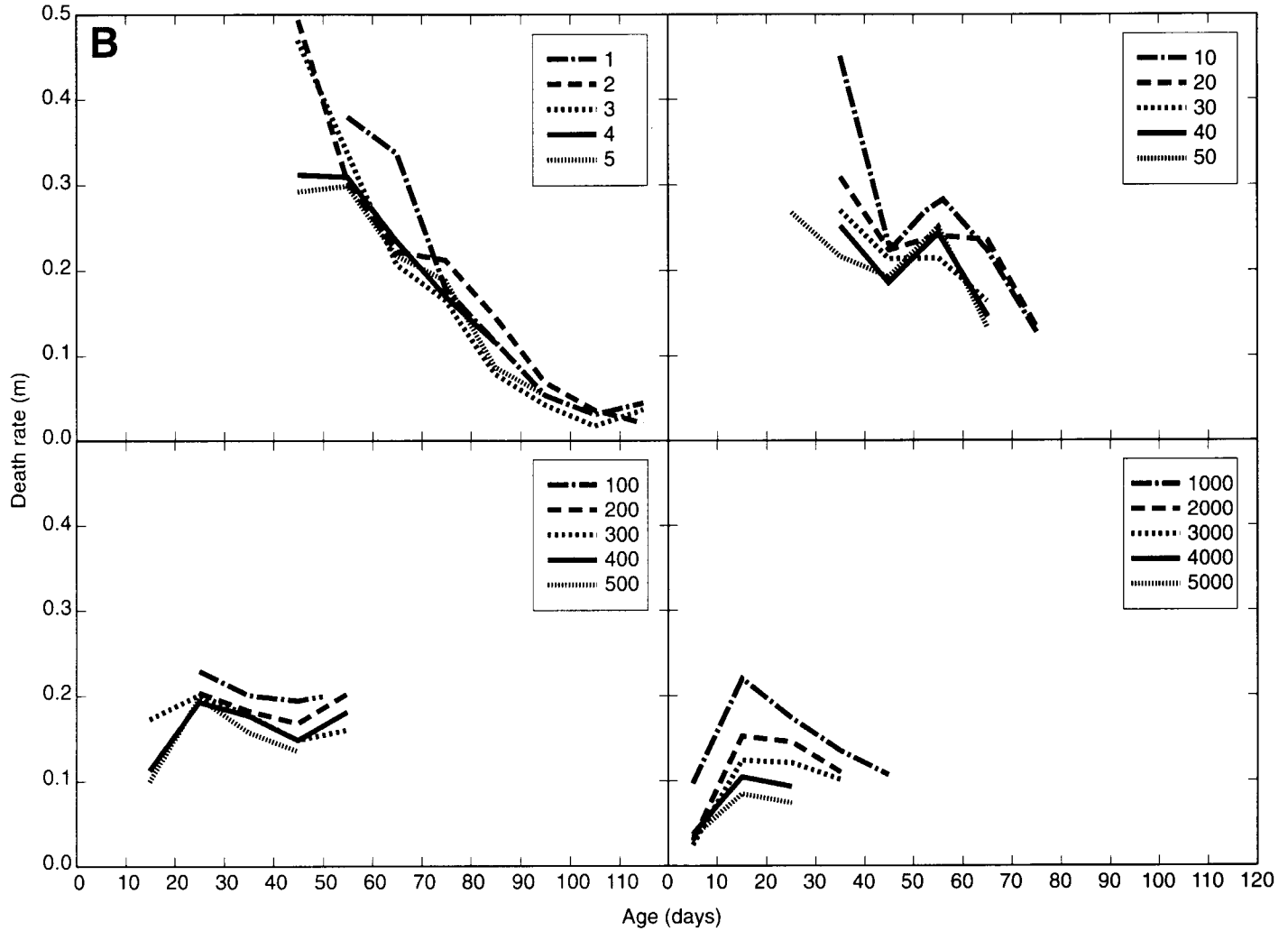
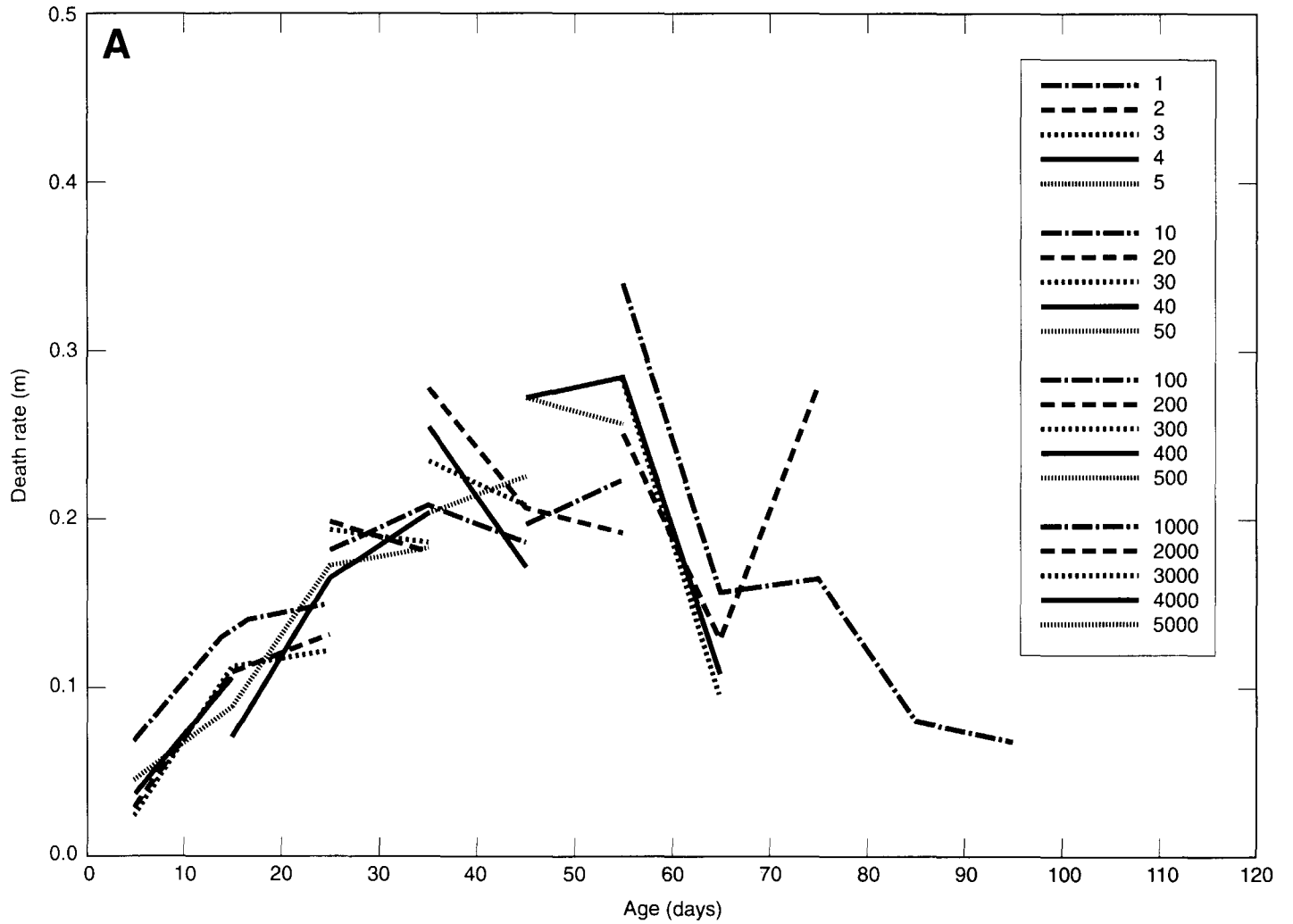


FIG. 8. Daily age-specific death rates at specified densities from one fly per cage to 5000 flies per cage for (A) the density experiment; and (B) the density experiment and original 1.2 million medfly experiment combined. Along the lines plotted—i.e., holding density constant—death rates tend to increase at younger ages, roughly level off at middle ages, and decline at older ages. Values were estimated for successive 10-day intervals and are plotted at the midpoint of the relevant interval.



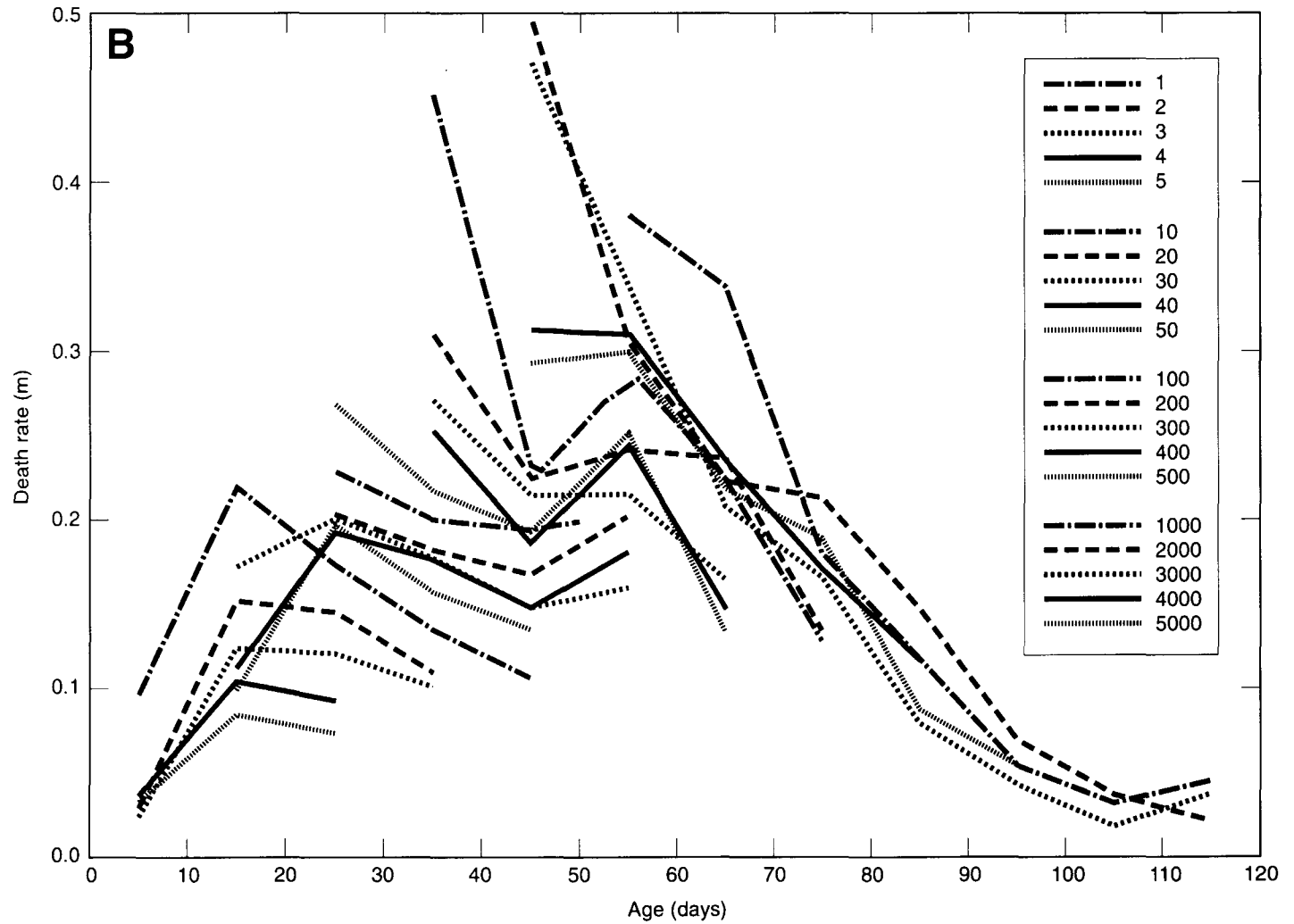


FIG. 9. Composite of age-specific death rates for (A) the density experiment; and (B) the density experiment and original 1.2 million medfly experiment combined.

TABLE 3. NUMBER OF NONEXTINCT CAGES AND MINIMUM, LOWER QUARTILE (L.Q.), MEDIAN, UPPER QUARTILE (U.Q.), AND MAXIMUM NUMBER OF SURVIVING FLIES AT THE START OF DAY X IN NONEXTINCT CAGES IN THE DENSITY EXPERIMENT (N = 210,000) AND THE ORIGINAL 1.2 MILLION MEDFLY EXPERIMENT

x	Density experiments						Original experiments					
	Cages	Min	L.Q.	Med	U.Q.	Max	Cages	Min	L.Q.	Med	U.Q.	Max
1	49	1054	2799	3157	5991	12,029	167	1770	6437	7518	8208	9708
10	49	692	2407	2605	4917	9755	167	800	5799	6924	7636	9357
20	49	350	930	1182	1517	3853	167	205	2430	3229	4498	7111
30	49	66	205	270	340	850	167	36	443	773	1541	3266
40	49	10	27	46	57	157	165	3	81	170	427	1265
50	49	1	5	8	13	30	162	1	17	35	92	402
60	42	1	1	2	3	14	152	1	4	7	16	99
70	30	1	1	1	2	6	111	1	1	3	5	34
80	13	1	1	1	1	2	77	1	1	3	3	15
90	7	1	1	1	1	1	54	1	1	1	2	8
100	3	1	1	1	1	1	41	1	1	1	2	5

Regression Model

The coefficient m^o —the estimated baseline death rate controlling for density and differential mortality—rises from day 5 until about day 35, then is roughly level until about day 45, and falls thereafter. The estimated value of a , the density coefficient, hovers around zero and is insignificant ($p > 0.05$) at nearly all ages. The value of b , the coefficient of differential mortality, is consistently positive, average roughly 0.6 up to

TABLE 4. CORRELATION BETWEEN PROPORTION DYING BEFORE START OF DAY X AND PROPORTION OF THOSE SURVIVING THAT DIE IN THE SUBSEQUENT 10-DAY PERIOD, FOR THE DENSITY EXPERIMENT, THE ORIGINAL 1.2 MILLION MEDFLY EXPERIMENT AND BOTH EXPERIMENTS COMBINED

Day x	Density experiment	Original experiment	Combined experiment
10	0.40 ± 0.12	0.52 ± 0.06	0.52 ± 0.05
20	0.80 ± 0.05	0.76 ± 0.03	0.77 ± 0.03
30	0.40 ± 0.12	0.69 ± 0.04	0.70 ± 0.03
40	0.48 ± 0.11	0.28 ± 0.07	0.28 ± 0.06
50	0.08 ± 0.14	-0.17 ± 0.08	-0.13 ± 0.07
60	-0.01 ± 0.15	-0.07 ± 0.08	-0.11 ± 0.07
70	-0.24 ± 0.17	-0.11 ± 0.09	-0.13 ± 0.08
80	-0.16 ± 0.27	0.07 ± 0.11	0.04 ± 0.11
90	^a	-0.03 ± 0.14	0.01 ± 0.13

Standard errors are given after the ± symbol. Correlations that are significant ($p < 0.05$) are in bold face.

^aFewer than 10 cages had any survivors.

day 70, and is significant ($p < 0.05$) at most ages up to day 40: cages with high past mortality tended to have high current mortality. The value of δ , which measures excess mortality in the density experiment relative to the original 1.2 million medfly experiment, is positive and significant ($p < 0.05$) during the first week, hovering around a value of 0.4, but is close to zero and insignificant ($p > 0.05$) thereafter. The value of 0.4 implies that death rates during the first week tended to be about 40% higher in the density experiment than in the original experiment. Mortality differentials of this magnitude, which are not unusual in replications of insect life-table experiments, are probably attributable to subtle differences in pupal quality and in such environmental factors as temperature, humidity, and food. For the model containing the additional term $\theta(x)\ln[m_i(x-1)/\hat{m}_i(x-1)]$, estimated value of $m^o(x)$, $a(x)$, $b(x)$, and $\delta(x)$ were very close to the values estimated in the simpler model. The coefficient $\theta(x)$ was consistently and significantly positive, varying around a value of about 0.4 up to about day 55 and a value of about 0.8 thereafter. Because persistent cage-specific factors affecting mortality are captured by $b(x)$, the positive values of $\theta(x)$ suggest that there are shorter term factors that elevate mortality on adjacent days. In addition to doing the analysis for single days, we estimated regression coefficients for 10-day periods, using the formula for $m_i(x)$ for Eq. (4), with $k = 5$. The results are similar to those reported above. We tested the hypothesis that the coefficients $m^o(x)$, $a(x)$, $b(x)$, and $\delta(x)$ were constant after day 10 and were only able to reject the hypothesis for m^o ($p < 0.05$). Estimated constant values for a , b , and δ are -0.03 , 0.46 , and -0.04 ; only the value of b is significant ($p < 0.05$).

DISCUSSION

One of the most significant results of this study is that the leveling off and decline in medfly mortality at older ages cannot be explained as a simple artifact of decreasing current density. The decline in medfly death rates occurred when densities were very low—from day 60 to day 100 fewer than 10 flies were typically alive in cages initially holding thousands of flies. Consequently, we hypothesize that other factors associated with heterogeneity among flies or cages are likely to be more important than declines in density in explaining the decline in mortality. Furthermore, we conjecture that even controlling for heterogeneity, age-specific mortality may decelerate at older ages. As Kowald and Kirkwood (1993) notes, “old flies lead quieter lives.” There may not be a simple link between activity levels and mortality: humans, for instance, tend to slow down with age, but death rates continue to rise. Finch (1990) reviews a variety of developmental and postmaturational influences on senescence.

A second important result of this study is that changes in the level of medfly crowding has a quantitative but not a qualitative effect on the age-specific mortality schedule. Virtually all aspects of the overall mortality patterns as well as relative differences in mortality and longevity between males and females were independent of initial density including: (1) age at which the leveling off of mortality occurred; (2) geometric rates of change in mortality with age; (3) relative differences in male and female longevity and mortality rates including mortality crossovers; and (4) mortality decline at advanced ages. These results suggest that increased crowding amplifies intrinsic age- and sex-specific patterns of vulnerability that are modulated by changes in age patterns of individual's reproductive biology. Crowding affects the rate of dying in a cohort but not the rate of aging, as measured by changes in the slope of mortality rates, inflection points, direction, and male-female differentials.

We believe that these main results have three important implications. The first implication is that it is probably impossible, even in theory, to eliminate crowding effects from an experiment. We found no evidence of an "optimal" density (Pearl *et al.*, 1927), although our range of densities was limited to only three. Even if an optimal density for medflies did exist and was known, the problem of interpreting density effects on mortality and longevity would still be present. We believe that density should be viewed simply as an environmental continuum, like temperature that can be adjusted and standardized but never eliminated. The fact of being in a captive, enclosed environment itself creates a density effect that is impossible to remove because it is an integral component of the controlled experiment. In general, understanding specific density effects may be less important for questions involving relative differences in life expectancy between two subgroups or treatments than for questions involving absolute differences.

The second implication is that life expectancy differences cannot be used as a proxy for changes in the mortality dynamics (see Carey *et al.*, 1992). The current analysis demonstrates that life expectancy differences caused by variation in densities reveal little about the nature of the mortality differences. Expectation of life or survival curves are summary measures and shed little light on deeper demographic and biological differences.

The third implication regards selection experiments. If the mortality differences we observed between medfly cohorts reared at different densities are similar to the underlying mortality differences in *Drosophila* density selection experiments (e.g., Graves and Mueller, 1993; Mueller *et al.*, 1993), then selection may be acting on traits that affect the mortality level and not on those that affect the mortality pattern. Understanding how selection acts on mortality in density experiments—whether on age-dependent or age-independent components—will provide more meaningful information than will knowledge of the consequences of mortality changes, as reflected in summary measures of life expectancy and survival.

In general, our results show that the arguments by Graves and Mueller (1993) that density effects account for the leveling off of mortality at older ages in medflies are unfounded. Curtsinger (1995) also argued that the Graves and Mueller (1993) density experiments on *Drosophila* were unpersuasive because: (1) the biology of the medfly is quite different than the biology of *Drosophila* and direct extrapolations from one species to another is questionable at best; (2) densities studied by Graves and Mueller (1993) were up to 120 times higher than in the medfly experiments; *Drosophila* at high densities were packed like a "swarm of bees" and, thus, it is not surprising that mortality was high at high densities; (3) Graves and Mueller (1993) ignored the leveling off of medfly mortality at older ages in cohorts of individuals maintained in solitary confinement; and (4) the Graves and Mueller (1993) *Drosophila* density experiments were quite small; two lines were studied at two densities with an average of about 275 flies per treatment. In contrast, the medfly studies consisted of 1.2 million individuals from the original study of caged medflies and 210,000 individuals maintained at 1 of 3 densities.

Our studies suggest that the overriding consideration in an experiment where controlling for density effects is important is to first ensure the consistency of initial densities among treatments and replicates. The biological effects of initial density may supercede the effects of the other types of density because the effects of the initial

density occur earlier than the others. Young, maturing flies are likely to be more vulnerable to the effects of crowding than older ones. Indeed, Pearl *et al.* (1927) found that the most marked effect of density of population was produced early in life. This "early, long-term impact" concept of the effects of initial density may be the biological reason that higher initial densities simply increased medfly mortality uniformly over all ages rather than altered the overall pattern and that the biological effects of both current and cumulative density on mortality are of secondary importance.

Density effects are virtually never considered in the context of human aging as is evident by the absence of this topic in almost all books on aging, senescence, and life span including those by Comfort (1979), Lamb (1977), Finch (1990), and Gavrilov and Gavrilova (1991). This lack of consideration of density effects in gerontology is surprising because it has long been known that crowding has a profound influence on factors that affect mortality rates including heightened incidence of social pathologies (Calhoun 1962) and social subordination (Christian 1970) in animal populations and increases in infection rates (Galle *et al.*, 1972), stress (Milgram 1970), and violence (Hawley 1972) in human populations. Indeed, the foundation of the seminal essay by Malthus (1799) on human populations concerns the ultimate effects of density on birth, death, and population homeostasis. Perhaps the most important direct connection of our findings to human mortality and aging is that our results on medfly mortality are consistent with a number of studies on mortality rates in high stress human populations showing that dire stress over a long period does not influence the rate of senescence during that time (Finch 1990). In our study we also found that the high stress caused by heightened cage densities did not influence the rate of change in mortality with age. Generally speaking, we believe that manipulations of density in experimental systems may eventually be viewed as one of the cleanest and most quantifiable methods available to gerontologists interested in studying the effects of stress on mortality and longevity.

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