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SENSITIVITY ANALYSIS OF POPULATION GROWTH RATES ESTIMATED FROM CLADOCERAN CHRONIC TOXICITY TESTS

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Abstract—Four variables—mean brood size, day of first reproduction, longevity and per capita rate of increase (r)—were compared in four 70-d chronic toxicity tests in which *Daphnia pulex* were exposed to cadmium or copper in continuous and pulsed exposures. With these data we asked the question: Is the population-level variable (r) more sensitive than the three organism-level variables (mean brood size, day of first reproduction and longevity) as an indicator of toxicant stress? We define two variables to be equally sensitive if, for both variables, differences between treatments and the control have statistical significance levels in the same probability range. In these four tests, none of the four variables was consistently the most sensitive. We also evaluated the sensitivity of r by simulating shorter test durations, delays in reproduction and less frequent observation schedules. Simulated test durations of less than 21 d produced biased underestimates of r and increased coefficients of variation of the estimates of r relative to the 70-d values; simulated 1- and 2-d delays in reproduction also produced biased underestimates of r. However, estimates of r computed for a simulated Monday-Wednesday-Friday observation schedule did not differ significantly from estimates of r computed for a daily observation schedule. We conclude that although the estimator of per capita rate of increase is not always the most sensitive statistic that can be computed from cladoceran chronic toxicity test data, it can be useful for evaluating apparently conflicting effects of pollutants on survival and reproduction, as occurred in the copper continuous-exposure toxicity test.

Keywords—*Daphnia pulex* Per capita rate of increase Survival Reproduction Cadmium Copper

INTRODUCTION

The National Research Council [1] recently emphasized the need to extend toxicological investigations beyond the organism level and predict effects of chemicals on populations, communities and ecosystems. For example, the per capita rate of increase of a population (r) can be estimated readily from data on the survival and reproduction of individual females in laboratory toxicity tests and can be used to predict effects of toxicants on population growth. Although biologists have estimated this parameter since the early 1900s [2,3], it has not been used commonly by toxicologists involved in either research or regulation.

Most toxicological studies that report estimates of r are based on data from one cohort of animals per toxicant exposure level and do not report standard errors or confidence intervals on estimates of r, thus precluding statistical inferences about estimated differences in r [4–8]. The few studies that report uncertainty (variance) in r have been based on replicate exposures of cohorts to a given toxicant level [9,10], which require additional time and effort to conduct and are not usually justified in routine toxicity testing. Because of this statistical inadequacy, the per capita rate of increase could not be used reliably (in a statistical sense) in the past as an indicator of toxicant stress. However, recently developed statistical methods for estimating uncertainty in r from data on individual cohorts of laboratory animals [11–13] constitute a major advance in the effort to incorporate population growth rates into evaluations of potential toxicant hazards.

In this article, we use two of those statistical methods, the jackknife and the bootstrap, to estimate means and standard errors of estimates of r for cohorts from four *Daphnia pulex* chronic toxicity tests. Then we compare the relative magnitude of change and uncertainty in r to the relative magnitude of change and uncertainty in three

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organism-level variables—day of first reproduction, mean brood size and longevity (day of death)—in order to address a long-standing, frequently debated question in environmental toxicology: Are population-level variables more sensitive than organism-level variables as indicators of toxicant stress? We also conduct a sensitivity analysis of the per capita rate of increase as it is affected by test protocol and potential toxicant stress. In that analysis, we (a) determine appropriate duration for *Daphnia* chronic toxicity tests, based on estimates of r and SE (r); (b) evaluate the effect of observation schedule on computational bias in estimates of r; and (c) simulate 1- and 2-d delays in reproduction to demonstrate the often underemphasized impact on estimates of r caused by a change in day of first reproduction.

DEFINITIONS

Three measures of sensitivity are often used, and confused semantically, when comparing statistics computed from the same data set. First, *relative magnitude of change* can be defined mathematically as

$$(\bar{x}_c - \bar{x}_t)/\bar{x}_c$$

where $\bar{x}_c$ is the estimate of the mean of variable x for the control population and $\bar{x}_t$ is the estimate of the mean of variable x for the treatment population. This is the most frequently used measure of sensitivity, yet it ignores variability in estimates of mean responses. For example, a large relative magnitude of change in $\bar{x}$ between the control and treatment might appear to be important, but it might not be statistically significant if variability in $\bar{x}$ is also relatively large.

Therefore, a second important measure of sensitivity is *relative variability*, which we define for this analysis as

$$\widehat{\mathrm{SE}}(\bar{x})/\bar{x}$$

where $\widehat{\mathrm{SE}}(\bar{x})$ is the standard error of the estimate of x. This measure is also commonly referred to as the coefficient of variation of the estimate of x.

Finally, we define *testing sensitivity* with respect to a given test statistic as the significance level (probability) associated with the comparison of a pair of estimated control and treatment statistics (e.g., means). For this analysis, we infer that variable x is as sensitive as variable y if differences between treatment statistics and the control statistic have significance levels in the same probability range for both variables: either $p > 0.05$, $0.05 > p > 0.01$ or $p < 0.01$. Probability values are determined from the following test statistic, which is used for pairwise comparisons:

$$(\bar{x}_c - \bar{x}_t)/[\sqrt{2} \cdot \widehat{\mathrm{SE}}(\bar{x})_p]$$

where $\widehat{\mathrm{SE}}(\bar{x})_p$ is the pooled estimate of the standard error of the estimate of x, as described in the following section. This test statistic is proportional to the ratio of the other two sensitivity measures.

In this article, we refer to all three types of sensitivity, but final interpretations will be based on testing sensitivity.

MATERIALS AND METHODS

Cladoceran chronic toxicity tests

Survival and fecundity data were taken from a previously published laboratory toxicity study by Ingersoll and Winner [14]. In that study, effects of continuous and pulsed exposures to cadmium and copper were determined for the aquatic invertebrate *Daphnia pulex*. Briefly, tests were started with neonate females (<24 h old) and continued for up to 70 d. Animals were maintained in 40 ml of reconstituted laboratory water in individual beakers at 20°C under a 16-h light:8-h dark photoperiod. The survival and reproduction of 8 or 10 females per cadmium or copper concentration and in controls were monitored daily. New offspring were removed daily from the beakers, and adults were transferred to fresh exposure solutions every 3 d. Although survival and reproduction of control and exposure cohorts were analyzed, estimates of per capita rates of increase were not reported because the statistical uncertainty surrounding those values was not estimated.

In this article, we reanalyze the data of Ingersoll and Winner [14] for four separate tests conducted under the following toxicant exposure regimes (test numbers as designated in ref. 14): continuous cadmium (Test 3), pulsed cadmium (Test 2), continuous copper (Test 2) and pulsed copper (Test 1). First, we estimate per capita rates of increase and standard errors of estimates using the statistical procedures described below. Then we (a) determine significance levels of differences between estimates of r for controls and treatments within each test, and (b) compare those results with the corresponding significance levels of differences between estimates of three organism-level indexes of survival and reproduction reported by Ingersoll and Winner [14] to evaluate the sensitivity of the per capita rate of increase as an indicator of toxicant stress. Those three organism-level indexes of survival and reproduction are longevity

(number of days lived), mean brood size (total number of live offspring born per number of broods produced by females surviving to reproduce at least once) and day of first reproduction.

Population growth rate calculations

Per capita rates of increase were estimated according to the Euler equation [15]:

$$1 = \sum_{x=0}^{\Omega} e^{-rx} l_x m_x \qquad (1)$$

where r is the per capita rate of increase for the cohort (d^{-1}), x is the age class (days; 0, 1, 2,..., Ω), Ω is the oldest age class in the population, l_x is the probability of surviving to age x and m_x is the fecundity at age x. Because this calculation involves a summation over several age classes, r cannot be isolated on one side of the equation to provide a closed-form, algebraic solution. Instead, iterative calculations must be performed to determine a value for r that satisfies Equation 1.

For the computations reported in this article, we used a half-interval iteration algorithm [16] programmed onto a Control Data Corporation Cyber 760 computer. Approximations used to initiate the iteration procedure and criteria used to terminate iterations are described by Meyer et al. [13]. (Copies of the FORTRAN V computer program are available from the authors.)

Statistical analyses

We estimated standard errors of r [$\widehat{SE}(r)$] and adjusted for mathematical bias in mean values of r using two computer-intensive statistical techniques, the jackknife [17] and the bootstrap [18]. Meyer et al. [13] demonstrated previously that both techniques reduced bias when compared with full-sample estimates of r (where a full-sample estimate is the value computed from the original test data using Eqn. 1) and provided reliable 95% confidence intervals on r based on repeated subsampling of computer-generated hypothetical cladoceran populations. Since estimates of r and $SE(r)$ for data sets analyzed in this article were almost identical using both techniques, and since the jackknife technique requires considerably less computer time than the bootstrap [13], we present results from only the jackknife technique in this article. Details of the jackknife, a recommended bias adjustment procedure for the bootstrap and corresponding methods for estimating 95% confidence intervals on r for cladoceran cohort data are described by Meyer et al. [13].

For each variable analyzed (day of first reproduction, mean brood size, longevity and per capita rate of increase), pairwise comparisons were made between treatment and control values using Dunnett's multiple comparison procedure [19]. Since r and $\widehat{SE}(r)$ values are computed for entire cohorts and not for individuals, we could not use analysis of variance to evaluate differences between estimates of control and treatment r values. Instead, we computed a pooled standard error from all of the control and treatment $\widehat{SE}(r)$ values in a test, as follows:

$$\widehat{SE}(r)_p = \sqrt{\frac{1}{k} \cdot \sum_{i=1}^{k} [\widehat{SE}(r)_i^2]}$$

where k is the number of control and treatment levels tested. Pairwise comparisons were then computed as follows:

$$t^* = \frac{r_c - r_t}{\sqrt{2} \cdot \widehat{SE}(r)_p}$$

where r_c is the estimate of control r, and r_t is the estimate of treatment r. The computed value of t^* was then compared with tabulated two-tailed values of Dunnett's t at $\alpha = 0.05$ and 0.01 [20] to determine the significance level associated with the pairwise difference. Pairwise comparisons for the other three variables were computed similarly so that significance levels of all four variables could be compared on an equal basis.

Homogeneity of variances was tested by Hartley's F-max test [21] using the ratio $F_{max} = \max_i[\widehat{SE}(r)_i^2]/\min_i[\widehat{SE}(r)_i^2]$. If the homogeneity of variance assumption was rejected at $\alpha = 0.01$ in any test (e.g., longevity comparisons in continuous cadmium exposure; Fig. 1), pairwise comparisons between the control and each treatment level were made using a pooled standard error of only the control and that treatment level [21].

Simulations

The appropriate duration and observation schedule for cladoceran chronic toxicity tests has recently been questioned [9,22]. To address this problem, we truncated the 70-d data sets for four control cohorts of *D. pulex* so that the data were analyzed through days 7, 9, 11, 14, 21, 28, 35, 42, 49, 56 and 63. We compared per capita rates of increase estimated from these truncated survival and fecundity schedules with those for the full 70-d tests to determine if estimates of r and $SE(r)$ stabilized as simulated test duration increased.

Additionally, we collapsed the daily observations on survival and reproduction in those same control cohorts into a Monday-Wednesday-Friday (MWF) schedule. This procedure is similar to that used by human demographers, who block groups of years (e.g., 0–5, 6–10, 11–15 years, etc.) to simplify calculations of population growth rates [23]. Moreover, it simulates the MWF observation and transfer protocol that is currently recommended for *D. magna* chronic toxicity tests [24]. For these simulations, the age class (x) in Equation 1 was assumed to be the midpoint of the age interval (e.g., x for the interval from day 4 to day 7 was 5.5). Per capita rates of increase estimated for these collapsed data sets were then compared with r values estimated previously for the daily observation schedule.

Finally, day of first reproduction is often overlooked as an indicator of toxicant stress. Therefore, we simulated the effect of 1- and 2-d delays in reproduction that might be caused by exposure to a toxicant. Data for the same control cohorts analyzed above were adjusted so that each brood was born 1 or 2 d later than actually observed.

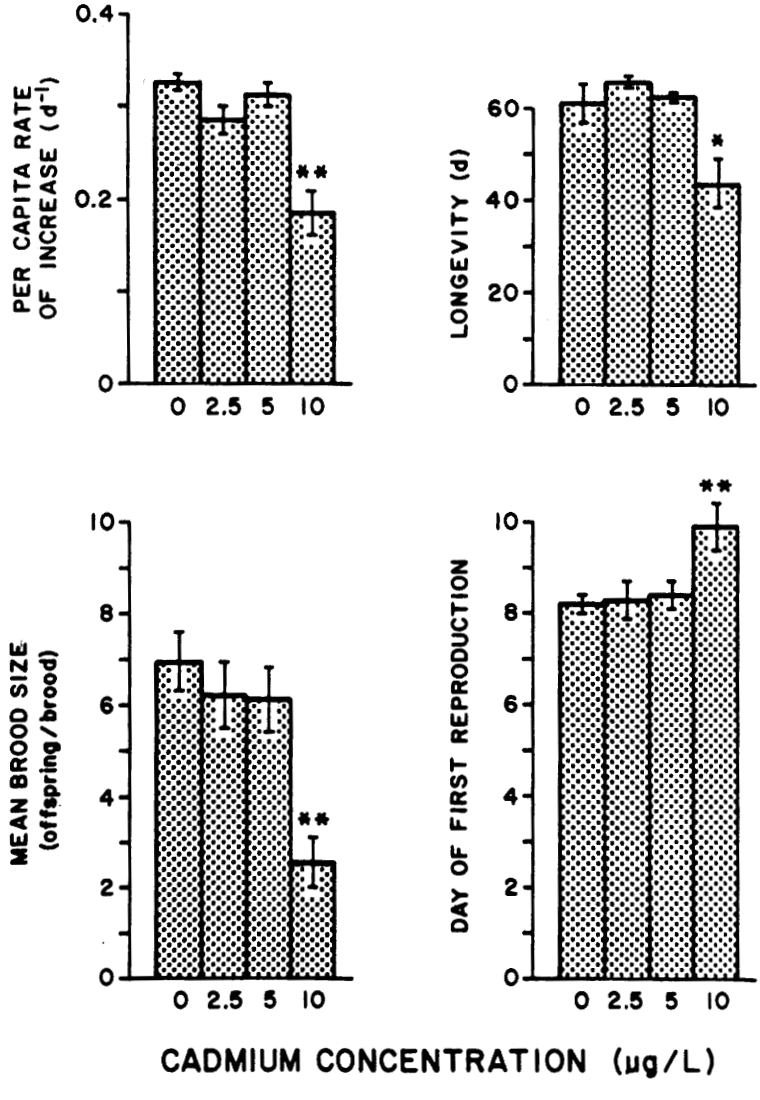

Fig. 1. Per capita rate of increase, longevity, mean brood size and day of first reproduction for control and exposure cohorts of *Daphnia pulex* in 70-d cadmium continuous-exposure toxicity test. Mean values ±1 SE are shown. Significance levels for differences from control values: $*p < 0.05$; $**p < 0.01$.

However, we did not alter brood sizes or longevity of adults. Estimates of r for cohorts with simulated delayed reproduction were then compared with estimates of r for the original cohorts to test for magnitude and significance of changes.

RESULTS

Estimates of per capita rate of increase, longevity, mean brood size and day of first reproduction for *D. pulex* exposed to cadmium or copper in continuous and pulsed exposures are compared in Figures 1, 2, 3 and 4. In two of the four tests (continuous cadmium and pulsed copper exposures; Figs. 1 and 4), the per capita rate of increase was as sensitive an estimator of toxicant stress as mean brood size and day of first reproduction (i.e., the probability ranges for the significance level of differences between control and treatment responses for the three variables were the same in both tests). Longevity was the least sensitive estimator in those two tests. Estimates of r were more sensitive than estimates of mean brood size in the continuous copper exposure (Fig. 2), but they were not as sensitive as estimates of longevity. There were no significant differences in day of first reproduction and in mean brood size in the con-

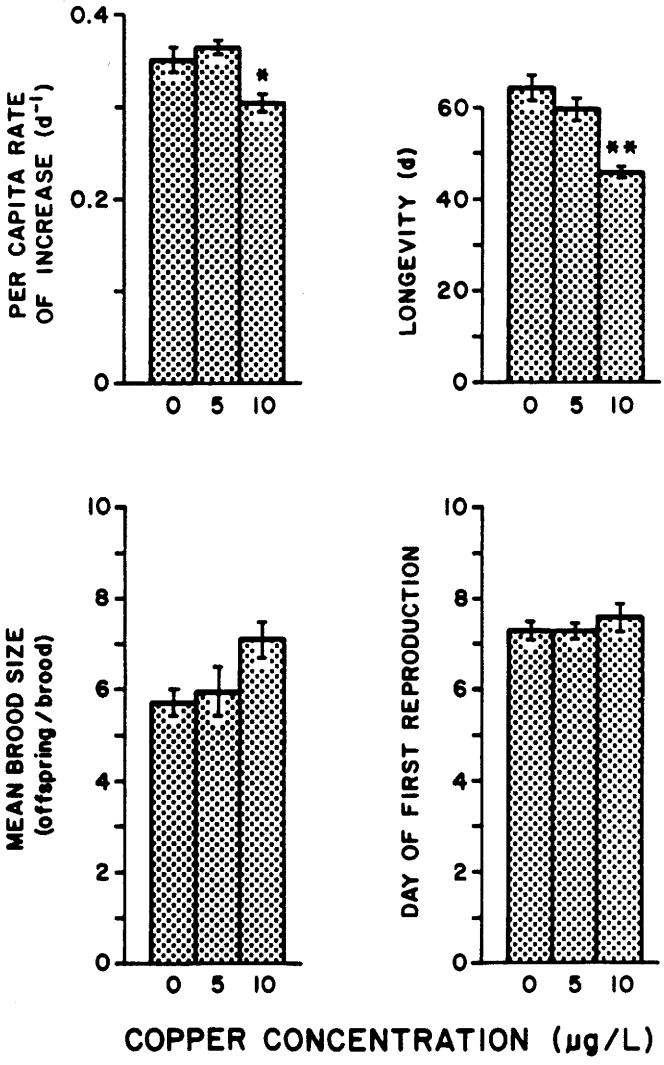

Fig. 2. Per capita rate of increase, longevity, mean brood size and day of first reproduction for control and exposure cohorts of *Daphnia pulex* in 70-d copper continuous-exposure toxicity test. Mean values ±1 SE are shown. Significance levels for differences from control values: $*p < 0.05$; $**p < 0.01$.

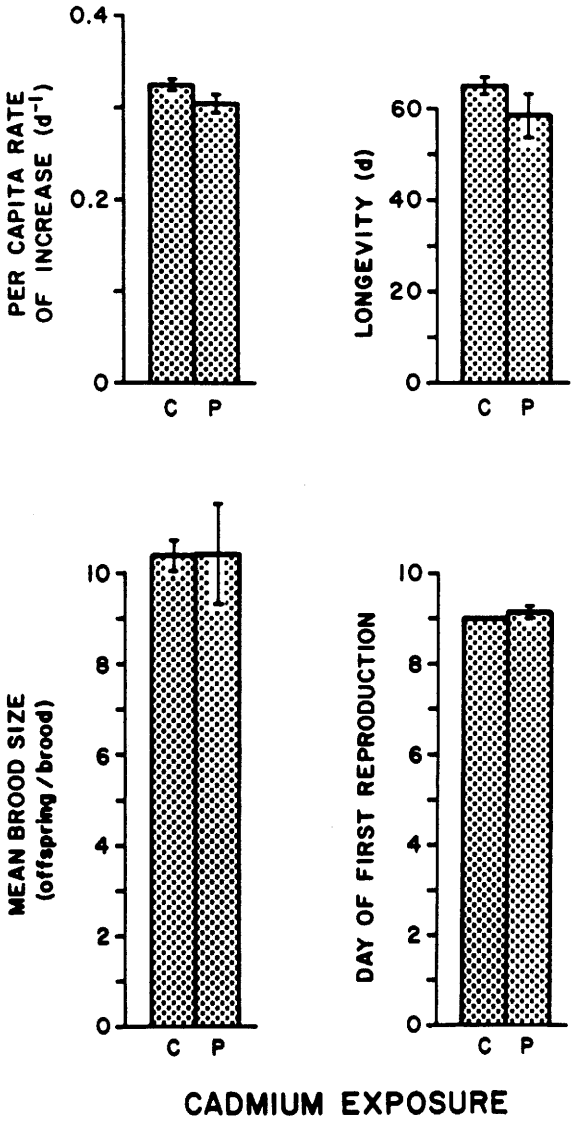

Fig. 3. Per capita rate of increase, longevity, mean brood size and day of first reproduction for control (C) and exposure (P) cohorts of *Daphnia pulex* in 70-d cadmium pulsed-exposure toxicity test, in which pulsed-exposure cohort was exposed to 100 μg Cd/L for 70 min daily. Mean values ±1 SE are shown. No exposure values were significantly different from control values.

tinuous copper exposure (Fig. 2), and there were no significant differences in any of the four variables in the pulsed cadmium exposure (Fig. 3). The coefficients of variation of estimates of r tended to be less than the coefficients of variation of estimates of mean brood size and the coefficients of variation of estimates of longevity in all four tests, but relative magnitudes of change in r also tended to be less than relative magnitudes of change in mean brood size and longevity.

In all four tests, estimates of r and longevity tended to decrease as toxicant concentration increased, and estimates of day of first reproduction tended to increase as toxicant concentration increased (Figs. 1-4). Estimates of mean brood size decreased or remained the same as toxicant concentration increased in three of the tests (continuous cadmium, pulsed cadmium and pulsed copper exposures; Figs. 1, 3 and 4), but they increased (although not significantly, $p > 0.05$) as toxicant concentration increased in the continuous copper exposure (Fig. 2).

Figure 5 shows the effect on r of truncating a representative data set for a *D. pulex* control cohort from the original 70-d test duration to a simulated 7-d test. At day 28 for all four control

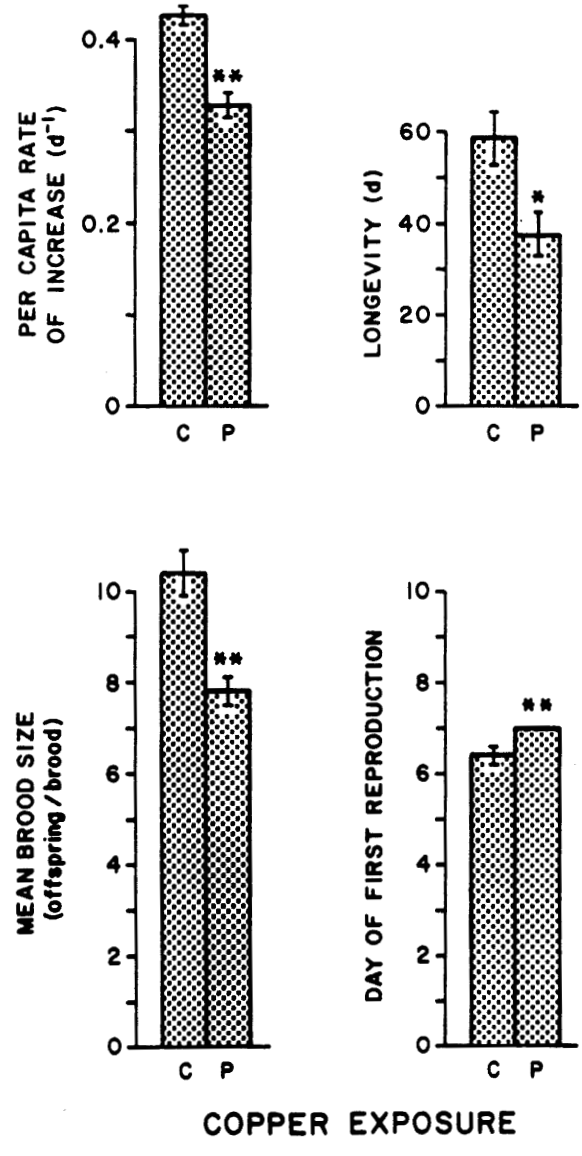

Fig. 4. Per capita rate of increase, longevity, mean brood size and day of first reproduction for control (C) and exposure (P) cohorts of *D. pulex* in 70-d copper pulsed-exposure toxicity test, in which pulsed-exposure cohort was exposed to 20 μg Cu/L for 360 min daily. Mean values ± 1 SE are shown. Significance levels for differences from control values: $^{*}p < 0.05$; $^{**}p < 0.01$.

cohorts, estimates of r rounded to three significant figures equalled the asymptotic 70-d estimates; at day 21, estimates of r were still within 1% of their respective 70-d estimates. Below day 21, the truncated estimates of r decreased rapidly and became negative between days 6 and 9, depending on the day of first reproduction in each cohort. Hence, after the first three to four broods, subsequent survival and reproduction did not change estimates of r appreciably. Figure 5 also shows that $\widehat{SE}(r)$ increased when the simulated duration of the test was decreased, a trend that was also evident in the other three control cohorts. Consequently, coefficients of variation of estimates of r [$\widehat{SE}(r)/r$] increased and statistical power decreased when simulated test durations were less than 21 d.

For the four control cohorts, estimates of r based on a simulated MWF observation schedule ranged from 2.5 to 4.6% less than estimates based on a daily observation schedule (Table 1). MWF estimates of r for the seven cadmium- or copper-exposed cohorts (not shown in Table 1) ranged from 3.3% less to 1.2% greater than daily estimates of r; no differences between paired MWF

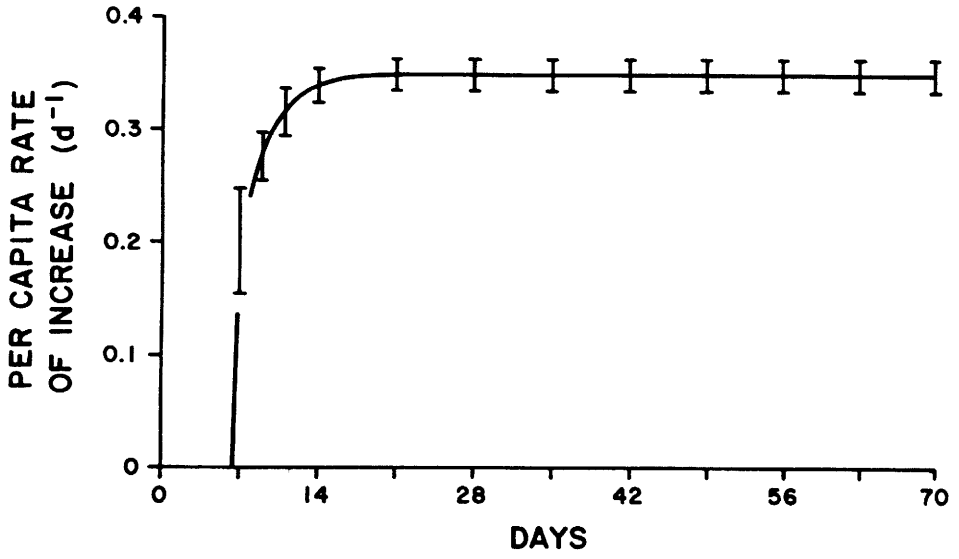

Fig. 5. Effects of test duration on estimates of per capita rate of increase for a control cohort of *D. pulex*. *r* values were computed from survival and fecundity schedules truncated at days 7, 9, 11, 14, 21, 28, 35, 42, 49, 56, 63 and 70. Mean values ±1 SE are shown.

and daily estimates of *r* were significant ($p > 0.05$). Differences in standard errors of *r* between the two observation schedules were small (≤21%) in 10 of the cohorts and relatively large (56%) in only one cohort.

Estimates of *r* decreased by an average of 10.4 and 18.3%, respectively, when 1- and 2-d delays in reproduction were simulated for the four control cohorts of *D. pulex* (Table 2). Three of the four decreases in *r* were significant ($p < 0.01$) for the 1-d delays in reproduction, and all four were significant ($p < 0.01$) for the 2-d delays in reproduction.

DISCUSSION

Sensitivity of population growth rates can be evaluated at several levels of resolution. For example, Demetrius [25], Goodman [26] and Caswell [27] proposed equations for the partial derivatives of *r* and λ ($\lambda = e^r$) with respect to changes in survival and fecundity. For known or hypothetical effects of a pollutant on reproduction and survival, their deterministic formulae could be used to predict the relative magnitude of change in *r*. However, in this article, sensitivity is defined at a statistical inference level wherein relative magnitude of change and relative variability in estimates of parameters are considered. With this approach, several variables can be compared for significance of changes (sensitivities) due to pollutant exposure.

Our results for *D. pulex* exposed to cadmium and copper show that the per capita rate of increase, a population-level statistic that can be estimated from data on survival and reproduction in chronic toxicity tests, was as sensitive as mean brood size, a sublethal organism-level statistic that is often used to set criteria for toxicants. Longevity, another organism-level statistic that is used to set toxicant criteria, was more sensitive than per capita rate of increase in only one of the four

Table 1. Effects of observation schedule on estimates of per capita rate of increase (d^{-1}) for control cohorts of *Daphnia pulex*

	Observation schedule	
Cohort	Daily	Simulated MWF
1	0.328 (±0.009)	0.313[a] (±0.011)
2	0.351 (±0.014)	0.338[a] (±0.015)
3	0.427 (±0.010)	0.415[a] (±0.008)
4	0.326 (±0.005)	0.318[a] (±0.006)

MWF *r* values were computed by collapsing original data collected on a daily observation schedule into a simulated Monday-Wednesday-Friday observation schedule. *r* values expressed as mean value (±1 SE).
[a]Not significantly different from *r* value for daily observation schedule ($p > 0.05$).

Table 2. Effects of simulated delayed reproduction on estimates of per capita rate of increase (d^{-1}) for control cohorts of *Daphnia pulex*

	Simulated delay in reproduction (d)		
Cohort	0	1	2
1	0.328 (±0.009)	0.297[a] (±0.007)	0.272[a] (±0.006)
2	0.351 (±0.014)	0.313 (±0.011)	0.284[a] (±0.009)
3	0.427 (±0.010)	0.375[a] (±0.008)	0.337[a] (±0.006)
4	0.326 (±0.005)	0.297[a] (±0.004)	0.274[a] (±0.004)

r values for simulated 1- and 2-d delays were computed by adjusting data so that each brood was born 1 or 2 d later than actually observed, although brood sizes and longevity of adults remained the same. *r* values expressed as mean value (±1 SE).
[a]Significantly less than *r* value for no delay in reproduction within the same cohort ($p < 0.01$).

tests. Day of first reproduction, an organism-level statistic that is seldom used to set toxicant criteria, was never more sensitive than per capita rate of increase.

At first glance, per capita rate of increase might be assumed to be a highly sensitive estimator of toxicant stress because of its low relative variability, as measured by the coefficient of variation of *r* [13]. However, statistical sensitivity is a function of two components—relative variability and relative magnitude of change. Therefore, even though relative variability in *r* was usually smaller, mean brood size was usually as sensitive an estimator as *r* because its relative magnitude of change was usually greater than the relative magnitude of change in *r*. Longevity was more sensitive than *r* only when its variability was low (Fig. 2) relative to its variability in the other three tests.

Test duration influenced the estimates of per capita rate of increase considerably. For *D. pulex*, estimates of *r* calculated from data collected for at least 21 d were nearly unbiased estimators of per capita rates of increase, but estimates of *r* calculated from data collected in fewer than 21 d were biased underestimators. We have obtained similar results for laboratory cohorts of *D. magna* (J. Meyer and A. Boelter, unpublished data). Furthermore, McNaught and Mount [22] reported that estimates of *r* stabilized after three or four broods in *Ceriodaphnia dubia*, another cladoceran species used in laboratory toxicity tests; we have also observed similar results for *Ceriodaphnia* in our laboratory (D. Brookshire and J. Meyer, unpublished data). These results support Bertram's [9] conclusion, based on hypothetical data, that 30 d is a sufficient duration for *Daphnia* chronic toxicity tests if per capita rate of increase is used as the endpoint statistic. Our analysis agrees with the commonly accepted tenet that early mortality and reproduction have the greatest influence on population growth rates. Goodman [26] demonstrated mathematically that, in general, (a) the relative magnitude of change in *r* due to changes in fecundity will decrease as age increases if $r > 0$ or $r > \ln(\max p_x)$, where $p_x = l_{x+1}/l_x$ is the conditional probability of survival from age x to $x+1$; and (b) the relative magnitude of change in *r* due to changes in longevity will always decrease as age increases.

Based on our results, we speculate that the greatest relative magnitudes of change in *r* for *D. pulex* populations perturbed by toxicants will often occur during the first 14 d after birth (i.e., during the first two to three broods). However, increased coefficients of variation of estimates of *r* that occur when data are truncated at 14 d or less indicate that statistical power (the ability to infer significant differences between estimates of *r*—our criterion of testing sensitivity) might decrease if experiments are conducted for only 14 d, even though by Goodman's [26] criteria the sensitivity of *r* is greatest during this early period of life. A 21-d duration for *Daphnia* chronic toxicity tests (i.e., the first three to four broods) appears to provide nearly unbiased estimates of the per capita rate of increase and its variance.

The dependence of estimates of *r* on early survival and reproduction was illustrated in the continuous copper exposure test (Fig. 2). Although longevity decreased significantly at 10 μg/L Cu, mean brood size through day 70 increased. Thus, survival and reproduction indicated conflicting inhibitory and stimulatory organism-level effects of the toxicant. However, when reproduction for only the first 21 d of the test was considered, mean brood size decreased significantly at 10 μg/L Cu

(Fig. 6), indicating a potentially adverse toxicant effect during the early part of that 70-d test. Per capita rate of increase estimated from the 70-d data set showed a significant inhibitory effect at 10 μg/L Cu and remained unchanged by truncating the data at day 21. Therefore, 21-d analysis resolved the apparent dichotomy and demonstrated that copper inhibited early reproduction by *D. pulex* significantly ($p < 0.05$) and would have caused a significantly slower population growth rate ($p < 0.05$) even though later reproduction (days 21 to 70) in the 10 μg/L Cu exposure was higher than in the control.

Differences between estimates of r computed from a daily observation schedule and a simulated MWF observation schedule were small (<5%), and coefficients of variation of estimates of r were of approximately the same magnitude (Table 1). A small bias in estimates of r could produce a larger and apparently important bias in estimates of future population size using deterministic population growth models. However, we conclude that the currently recommended MWF adult transfer, feeding and observation schedule for *Daphnia* chronic toxicity tests [24] will not produce statistically significant computational bias if stochastic

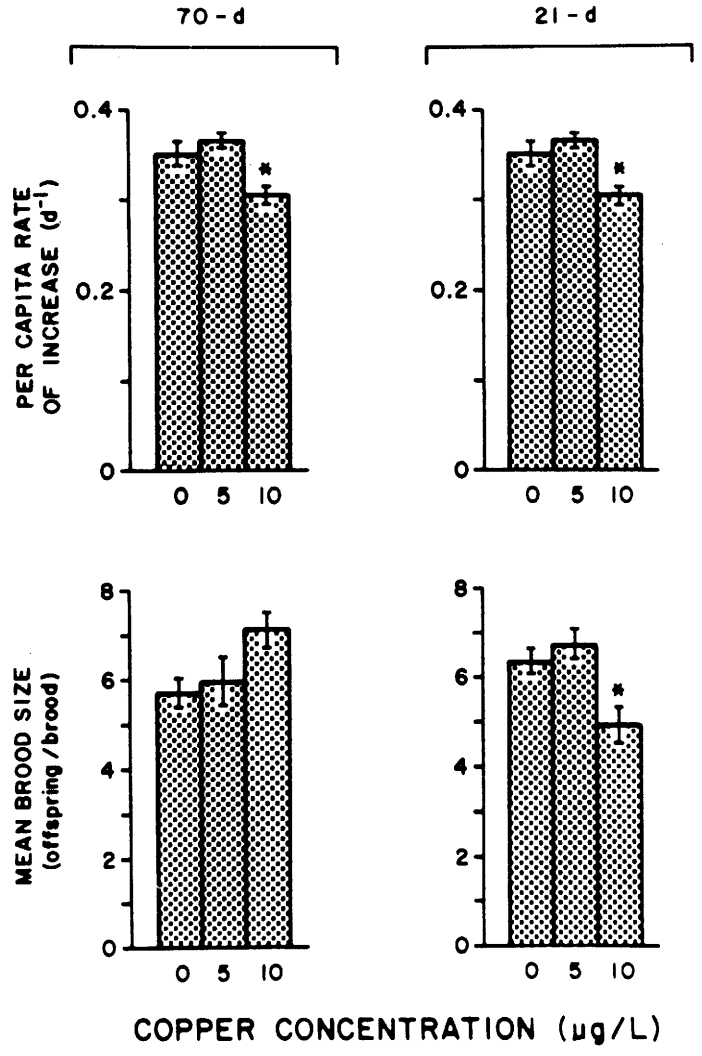

Fig. 6. Mean brood size and per capita rate of increase after 21 and 70 d for control and exposure cohorts of *D. pulex* in 70-d copper continuous-exposure toxicity test. Mean values ±1 SE are shown. Significance levels for differences from control values are: $*p < 0.05$.

population growth models, which incorporate uncertainty in estimates of r, are used. Still, we caution that the actual number of offspring produced in an MWF chronic toxicity test can be lower than the number predicted by collapsing reproduction from a daily observation schedule into a simulated MWF observation schedule because adult *Daphnia* must compete for food with offspring that remain in the test chamber for longer than 24 h (J. Meyer and A. Boelter, unpublished data).

Estimates of mean brood size (or total number of offspring) and longevity commonly are used to determine toxicant levels that cause adverse effects on cladocerans, yet the importance of delayed reproduction is often overlooked. For example, estimates of day of first reproduction increased significantly at the highest treatment level in two of the four toxicity tests analyzed in this study (Figs. 1 and 4). Furthermore, significant increases in estimates of day of first reproduction without concomitant decreases in estimates of brood sizes or longevity were reported for the copepod *Eurytemora affinis* exposed to dieldrin [7] and for the mysid shrimp *Mysidopsis bahia* exposed to mercury and nickel [8]. Demetrius [25] demonstrated that when $r > 0$ or $r > \ln(\max p_x)$, a decrease in age of maturation has a stronger effect on population growth rate than does an equivalent increase in longevity. Additionally, our simulated 1- and 2-d delays in reproduction caused large and significant decreases in estimates of r for control cohorts of *D. pulex* (Table 2). Reproduction may not always be delayed by toxicants, but these examples illustrate the importance of all three organism-level variables in determining population growth rates. Additionally, they show why it is important to collect neonates during a short time period ($\ll$24 h) immediately before starting a cladoceran chronic toxicity test and why it is important to adhere to a precise observation schedule (every 24 h) until all females have released their first brood.

We caution that a single statistic, such as per capita rate of increase, is not sufficient to fully describe population dynamics over a wide range of population densities and food availabilities. Our analysis is based on the assumption of exponentially increasing population growth ($r > 0$), which is reasonable only when population densities are relatively low and food availability is high. Under less ideal conditions, density-dependent competition for food could become more important and population growth rates could decline. Per capita rates of increase estimated using standard chronic toxicity test procedures, in which females are tested in separate beakers that contain excess food, might drastically overestimate growth rates of crowded populations. Hence, other parameters, such as carrying capacity, would have to be known in addition to the population's intrinsic rate of increase to more accurately predict the population growth rate [15]. Although in this study we have emphasized the importance of early survival and reproduction in exponentially growing populations, differences in reproduction and survival that occur late in life will be more important in stable or declining populations ($r \leq 0$) [28] and in some fluctuating environments.

In summary, although per capita rate of increase is not always the most sensitive statistic that can be estimated from cladoceran chronic toxicity test data, it can be extremely useful for evaluating apparently conflicting effects of pollutants on survival and reproduction and for determining the population-level significance of organism-level responses to pollutants. Gentile et al. [8] also concluded that r is a valuable and integrative measure of chronic toxicity, and they suggested that the effects of other ecological factors (e.g., predation) should be considered in combination with toxicant stress to predict population growth rates. Since our conclusions are based on a relatively small set of cladoceran chronic toxicity tests, more data sets should be analyzed to determine if the trends identified in these analyses are supported.

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