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LONGEVITY AND REPRODUCTION OF *CERIODAPHNIA DUBIA* IN RECEIVING WATERS

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Abstract—Seven-day tests with *Ceriodaphnia dubia* are commonly used to estimate toxicity of effluents or receiving waters but can sometimes yield no toxicity outcomes even if pollutants are present. We conducted two sets of full life-cycle tests with *C. dubia* to (1) determine whether tests with longer exposure periods to low concentrations of contaminants in ambient water might reveal evidence of toxicity that could not be discerned from 7-d tests and (2) determine the relative importance of water quality versus food as factors influencing *C. dubia* longevity and reproduction. In the first set of tests, *C. dubia* was reared in diluted mineral water (a negative control), water from a stream impacted by coal fly-ash, or water from a retention basin containing sediments contaminated with mercury, other metals, and polychlorinated biphenyls. The second set of tests used water from the retention basin only. Before testing, though, water in the second set of tests was either filtered or not filtered, and food was either added or not added. *Ceriodaphnia dubia* longevity and reproduction did not differ much among the three water types in the first set of tests, but both longevity and reproduction were strongly affected by the filtering and food-addition treatments in the second set of tests. Thus, *C. dubia* appeared to be relatively insensitive to general water quality factors but sensitive to food-related factors. In five of the six full life-cycle tests, lifetime reproduction by *C. dubia* could not be reliably predicted from reproduction data from the first 7 d of testing ($R^2 < 0.35$, by regression analysis). The increase in predictability of lifetime reproduction of *C. dubia* as a function of test duration also differed among water types in the first set of tests and among treatments in the second set of tests. Thus, it may not be possible to reliably extrapolate the results of 7-d tests with *C. dubia* to longer time scales.

Keywords—*Ceriodaphnia dubia* Ambient toxicity testing Daphnid ecology Biomonitoring

INTRODUCTION

The freshwater microcrustacean *Ceriodaphnia dubia* can be used in short-term standardized tests to estimate the acute or chronic toxicity of chemicals [1,2], effluents [3,4], and freshwater receiving systems [5–8]. The use of this animal as a representative aquatic organism in such tests is justified in part because it has a widespread geographic distribution and holds an intermediate position in pelagial or planktonic food webs: it consumes algae and detritus and in turn is consumed by various predators. *Ceriodaphnia* is also convenient to use in such tests because it is sensitive to various toxic chemicals, is easily reared under laboratory conditions, and has a moderately short life cycle [9].

Knowledge about the basic biology and life-history attributes of organisms used for toxicity testing can be helpful in several ways. First, it can be used to develop simpler or more effective testing or culturing protocols or to more accurately define limitations of the test method. Second, knowledge about the response patterns of the organisms to various environmental factors, such as pH, can allow the investigator to more accurately interpret the results of toxicity tests [10]. Third, the results of short-term toxicity tests sometimes are extrapolated to predict the responses of other species to the contaminant in question or incorporated into predictive models to estimate the effects of longer-term exposures to a contaminant when laboratory toxicity test results are used to estimate ecological risk. The accuracy or limitations of extrapolated estimates of risk that use the results of *C. dubia* tests might be assessed more clearly by a better understanding of the basic biology of these organisms in toxicity testing situations.

In this study we report the results of two sets of full life-cycle tests with *C. dubia* in which the animals were reared in water from various streams, with and without treatments to manipulate food. Our central objective was to determine whether longer exposure to water from two streams known to contain low concentrations of contaminants with a tendency to bioaccumulate might unveil adverse physiological effects not detectable using conventional 7-d ''minichronic'' test procedures.

MATERIALS AND METHODS

The test methods used in this study were similar to the methods of the U.S. Environmental Protection Agency (EPA) described by Weber et al. [3] and identical to those used for other studies conducted in our laboratory [11–13] with two exceptions. First, instead of using 10 replicates (one daphnid per beaker in 15 ml of water), we started with 50 replicates (one daphnid per beaker in 15 ml of water). Second, rather than ending a test after 7 d, we continued daily transfers of the animals (and daily counts of their offspring) until the last adult animal had died.

Two sets of full life-cycle tests were conducted. The first was started on July 27, 1990, and lasted 41 d. In this set of tests, *Ceriodaphnia* neonates <24 h old (differing <4 h in age) were used to test three types of water: control (25% diluted mineral) water, water from Lake Reality outfall (LR-o), and water from McCoy Branch. Lake Reality is a 1-ha im-

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Table 1. Water quality parameters (mean ± SD) for two sets of life-cycle tests with *Ceriodaphnia dubia*[a]

Test set	Water source	pH	Conductivity (μS/cm)	Alkalinity (mg/L)	Hardness (mg/L)
1	Control water	8.17 ± 0.06	177 ± 5	65.1 ± 2.9	84.2 ± 5.5
	McCoy Branch	8.00 ± 0.10	343 ± 24	163.2 ± 8.7	186.7 ± 6.8
	Lake Reality outfall	8.41 ± 0.28	433 ± 40	111.8 ± 6.2	192.5 ± 8.5
2	Lake Reality outfall	8.08 ± 0.15	487 ± 175	110.6 ± 23.1	199.8 ± 43.1

[a] Means and SDs for ambient water quality parameters in the first and second set of tests are for 17 and 26 observations, respectively; means and SDs for control water parameters are for 12 observations.

poundment of East Fork Poplar Creek located near the U.S. Department of Energy's Oak Ridge Y-12 Plant in eastern Tennessee. East Fork Poplar Creek near Lake Reality has been well characterized biologically and toxicologically [7,14–17]; the sediments in this stream contain various pollutants, including mercury and polychlorinated biphenyls (PCBs) [14]. McCoy Branch, a first-order stream located 5 km southwest of Lake Reality, has historically been contaminated with fly ash. The fly ash originated from a coal-fired power plant at the Oak Ridge Y-12 Plant and was sluiced to a settling basin near the stream's headwaters. Contaminants of concern in McCoy Branch stream include arsenic and selenium [18,19].

The second set of tests was started on March 9, 1993, and lasted 62 d. In this set of tests, *Ceriodaphnia* neonates $<$24 h old (differing $<$4 h in age) were tested in water from one site only (LR-o). This test, though, used two treatments: Grab samples of water freshly collected from LR-o were either filtered (0.5 μm pore size, glass-fiber filters) or not filtered, and the daphnids within each filtration treatment were either not fed or fed (100 μl of food per beaker per day, when the water was renewed).

In both sets of tests, food, when supplied, consisted of a 100-μl volume of a standard mixture of yeast, fermented trout chow, and cerophyll (YTC; containing 2.3–2.8 mg/ml solids), plus algae (*Selenastrum capricornutum*; 3.0–3.5 $\times 10^7$ cells/ml) [3] added daily to each beaker. The YTC was mixed 1:1 by volume with the algal culture before use. In both sets of tests, water samples from the two ambient sites (McCoy Branch and/or LR-o) were collected as grab samples three times weekly (Monday, Wednesday, and Friday) between 08:00 and 10:00 h. The freshly collected grab samples were analyzed for pH, conductivity, alkalinity (EPA method 130.1) and hardness (EPA method 130.2). The water samples were stored at 4°C, and portions were warmed to testing temperature (25 ± 1°C) daily before use.

Data from both sets of tests were summarized and analyzed using the Statistical Analysis System (version 6.04 for personal computers [20]). Mean daily reproduction was calculated as the number of *C. dubia* offspring produced each day divided by the number of females alive on that day. Reproductive synchrony was inspected by plotting the percentage of live adult daphnids that produced any offspring on each day of each test. Mean daily brood size was computed by dividing the total number of offspring produced, on any day, by the number of daphnids producing any offspring on that day, for each treatment or site. We had no a priori hypotheses about how water from different sites, or treated by filtering or manipulating food, might affect reproductive synchrony or daily mean brood size in relation to longevity.

In the first set of tests, analysis of variance (ANOVA) was used to test for differences in various reproductive parameters of the daphnids in response to water type. In the second set of tests, ANOVA was used to test for effects of the filtering versus the nonfiltering treatment, and the food versus the no-food treatment, on reproductive parameters of *C. dubia*. Simple correlation analysis was used to inspect associations between life span, maximum brood size, and the total number of offspring produced per female. We used regression analysis to estimate how well the total number of offspring produced per female by the end of the 7th, 8th, 9th, 10th, etc., to the last day of life, for any treatment combination, could reliably predict lifetime reproduction. To increase comparability of results, these regressions used data only from *C. dubia* that lived for at least 15 d for each set of tests.

RESULTS

Water quality factors

Data for pH, conductivity, alkalinity, and hardness of water used in the two sets of tests are summarized in Table 1. No rainfall occurred during the first testing period, so day-to-day variation in water quality factors for McCoy Branch and LR-o was low. The mean pH of water from LR-o was elevated slightly (8.4) relative to McCoy Branch (8.0) and more variable than that for water from McCoy Branch because upper East Fork Poplar Creek is nutrient enriched and very productive. During the second set of tests, conductivity increased briefly in East Fork Poplar Creek on March 16 (to 989 μS/cm), but alkalinity, hardness, and pH values remained typical for that stream. During March 19–23, rainfall resulted in the dilution of conductivity, alkalinity, and hardness in LR-o water (to 174 μS/cm, 46 mg/L, and 82 mg/L, respectively). The conductivity increase and rainfall event together accounted for the more variable nature of water quality conditions in LR-o during the second set of tests compared with the first set of tests (Table 1). The pH, conductivity, alkalinity, and hardness of the control water used in the first set of tests varied little from batch to batch (Table 1).

Ceriodaphnia *longevity*

In the first set of tests, survival patterns of *Ceriodaphnia* in the three water types were similar (Fig. 1a). The (interpolated) length of time needed for 50% of the animals to die in control water, McCoy Branch water, and LR-o water was 23.7, 27.0, and 26.4 d, respectively; the maximum longevity of *C. dubia* in any of the waters was 41 d. The difference in *Ceriodaphnia* survival among the three water types increased gradually to a maximum (between days 17 and 24) before declining (Fig. 1a).

In the second set of tests, *C. dubia* survival patterns differed markedly among the four treatments (Fig. 1b). Maximum longevity was as short as 14 d for nonfed animals in filtered

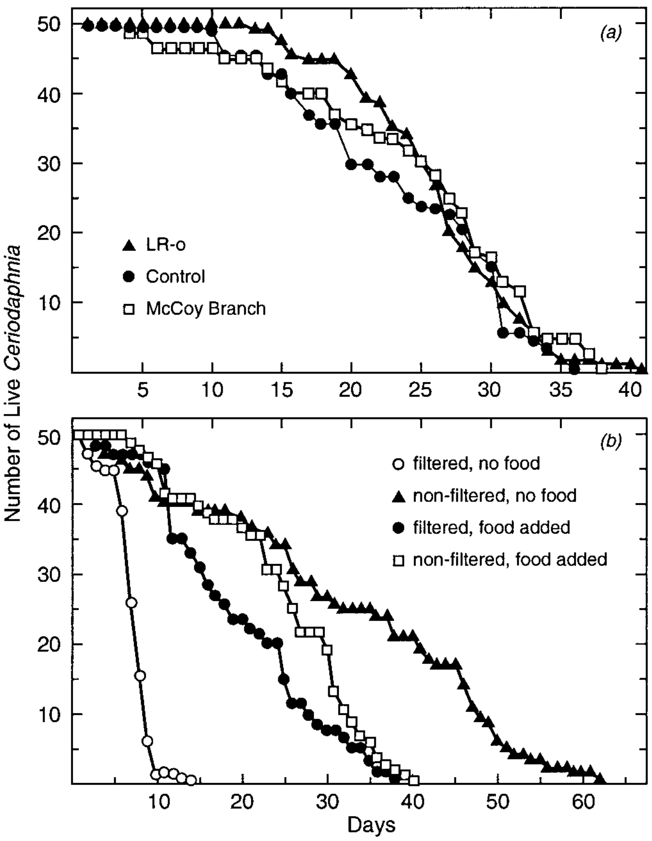

Fig. 1. Survival of *Ceriodaphnia dubia* in water from McCoy Branch, Lake Reality outfall (LR-o), and control water in the first set of life-cycle tests (**a**) and in water from LR-o in the second set of life-cycle tests (**b**).

LR-o water and as long as 62 d for nonfed daphnids in nonfiltered LR-o water. The (interpolated) length of time needed for 50% of the daphnids to die ranged from 7.2 d for nonfed animals in filtered LR-o water to 32 d for nonfed animals in nonfiltered LR-o water (Fig. 1b).

Ceriodaphnia *reproduction synchrony*

In the first set of tests, mean daily reproduction of *C. dubia* occurred predominately as an extended series of more or less regularly spaced pulses (Fig. 2a). The pulses generally were 2 d in duration and were separated from one another by 1-d gaps. As many as 10 pulses in reproduction were evident for *C. dubia* reared in control water, but in LR-o water only four to five pulses were evident. Reproduction of the daphnids tended to be synchronous, particularly during the first 15 to 20 d of the tests: >80% of the daphnids released offspring on days 4 to 5, 7 to 8, 10 to 11, 13 to 14, etc. However, even on the low-production days between reproductive pulses, 10 to 30% of the *C. dubia* released offspring.

In the second set of tests, the reproductive pulses of *C. dubia* were less regular than those noted in the first set of tests. Reproductive synchrony also was affected by the filtration and food-addition treatments (Fig. 2b). In nonfiltered LR-o water with no food added, first reproduction was delayed until day 6. The reproductive synchrony of *C. dubia* in this treatment combination was also lower than in the two treatments, both of which included the addition of food. In nonfiltered LR-o water with food added, the proportion of the daphnids reproducing each day generally exceeded the proportion of daphnids reproducing each day in the filtered LR-o water to which food was added, especially after day 20 (Fig. 2b).

Ceriodaphnia *brood sizes*

The mean total number of offspring per daphnid, mean largest brood per daphnid, mean number of broods per daphnid, and mean number of offspring per brood, computed from full life-span data for *Ceriodaphnia* in both sets of experiments, are summarized in Table 2.

In the first set of tests, overall mean brood sizes of *Ceriodaphnia* among the three water types were similar (Table 2). These means, though, were computed by pooling through time and thus ignored day-to-day differences in brood size. To examine time-related changes in mean brood size of *Ceriodaphnia* (and change in standard deviation [SD] of mean brood size), we plotted mean brood size and SD of the mean brood size versus time, pooling data for the three types of water. For the first set of tests, pooling was justified on the basis of similarity in survival curves (Fig. 1a) and total reproduction (Table 2). Mean brood size increased from about 5 offspring per brood on day 4 to a maximum of about 16 offspring per brood (day 11), then declined (Fig. 3). Mean brood size in-

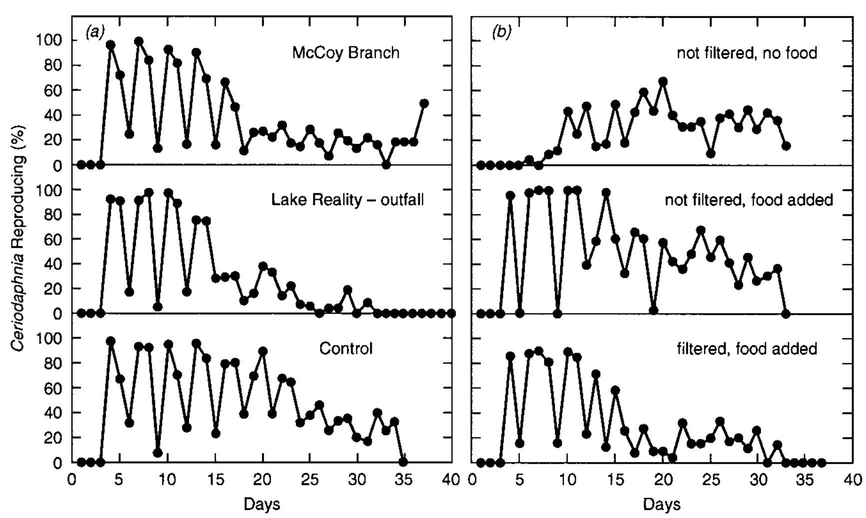

Fig. 2. Reproductive synchrony of *Ceriodaphnia dubia* in water from McCoy Branch, Lake Reality outfall (LR-o), and control water in the first set of life-cycle tests (**a**) and in water from LR-o in the second set of life-cycle tests (**b**).

Table 2. Mean life span and means (±SE) for various reproductive parameters of *Ceriodaphnia dubia* in full life-cycle tests with water from various sources and/or water from one source (Lake Reality outfall), but either filtered or not filtered, with food either added or not added

Water source	Treatment	Mean life span (d)	Total offspring per female (*n*)	Offspring in largest brood (*n*)	Broods (*n*)	Offspring per brood (*n*)
Control water	Not filtered, food added	23.1	119.6 ± 7.7	17.4 ± 0.5	12.4 ± 0.6[b]	9.8 ± 0.3
McCoy Branch		24.3	107.2 ± 6.6	18.1 ± 0.6	10.1 ± 0.5	10.7 ± 0.4
Lake Reality outfall		25.6	114.7 ± 4.0	19.7 ± 0.4[a]	9.7 ± 0.3	11.9 ± 0.3
Lake Reality outfall	Filtered, no food added	6.6	0	0	0	0
Lake Reality outfall	Filtered, food added	19.3	47.3 ± 3.4	10.7 ± 0.7	8.4 ± 0.5	6.9 ± 0.2
Lake Reality outfall	Not filtered, no food added	31.6	14.2 ± 1.4	2.9 ± 0.3	9.7 ± 0.7	1.8 ± 0.1
Lake Reality outfall	Not filtered, food added	24.3	86.1 ± 4.7	14.6 ± 0.3	12.0 ± 0.7	7.3 ± 0.2

[a] Significantly greater than mean brood size in control water or McCoy Branch water.
[b] Significantly greater than mean number of broods for *Ceriodaphnia* in McCoy Branch water or Lake Reality outfall water.

creased slightly during days 25 to 27, when 44 to 60% of the daphnids still survived (Fig. 1a). Except for one unusually high value on day 9, the SD values for daily mean brood size increased for the first 5 d of reproduction, from about 1.1 to 4.8; they then appeared to plateau, ranging from about 4 to 5.5. From Fig. 3, it seems clear that data from the first 7 d of testing are not necessarily representative of *Ceriodaphnia*'s reproductive potential: mean brood sizes on days 8, 10, and 11 were greater than the largest mean brood sizes for days 4 through 7, by 13 to 20%. The SD values for mean brood size for older *Ceriodaphnia* (e.g., days 15–29) also were greater than those for *Ceriodaphnia* broods produced by younger animals during the first 7 d of testing (Fig. 3).

In the second set of tests, mean brood size was consistently low for the nonfiltered, no-food-added treatment and was higher and more variable in the filtered and nonfiltered water to which food was added (Fig. 4). The presence of naturally occurring particulate matter in LR-o water appeared to augment brood size (note days 7–11 compared to the filtered, food-added treatment). In this set of tests, the ranges in SDs for mean brood size (by day, computed as described above) for the three treatments were 0.4 to 1.6 (nonfiltered, no-food-added treatment), 1.1 to 4.7 (nonfiltered, food added treatment), and 1.5 to 4.1 (filtered, food-added treatment).

Lifetime reproduction of Ceriodaphnia

In the first set of tests, total reproduction of *Ceriodaphnia* was not strongly affected by water source: the mean total number of offspring produced per daphnid ranged from 107.2 in McCoy Branch water to 119.6 in control water. In control water, the daphnids produced, on average, fewer offspring per brood (9.8 vs 11.9) but a larger number of broods (12.4 vs 9.7) compared to *Ceriodaphnia* reared in LR-o water (Table 2). Analysis of variance detected statistically significant differences in means for largest brood ($p \leq 0.01$) and in the number of broods produced per daphnid ($p < 0.001$) in response to water type. However, the proportion of variation in these reproductive parameters explained by water type was low (<12% in each case).

In the second set of tests, *Ceriodaphnia* in nonfiltered, food-augmented LR-o water produced only about 75% as many offspring as *Ceriodaphnia* in the nonfiltered LR-o food-augmented water that was used in the first set of tests (86.1 vs 114.7 offspring per female; Table 2). The filtering and food-addition treatments in the second set of tests both strongly affected *Ceriodaphnia* longevity and reproduction. When no food was added, the presence of naturally occurring particulate matter extended the mean life span of *Ceriodaphnia*'s from 6.6 to 31.6 d. In the second set of tests, the quantity and quality of the naturally occurring particulate matter also was great enough to sustain a modest level of reproduction (47.3 offspring per female) (Table 1). Analysis of variance showed that most of the differences in *Ceriodaphnia* reproductive parameters attributed to the filtering and food-addition treatments were highly significant. The proportion of variation explained by the treatments was large for mean brood size (86.6%), the largest brood produced during a female's life span (81.8%), and for the total number of offspring produced per female (71.7%). Mean fecundity rate was significantly affected both by the filtering ($p < 0.0001$) and the food-addition ($p < 0.001$) treatments, but the influence of the interaction term for these two treatments on the mean fecundity rate was not significant ($p = 0.175$).

Predictability of lifetime reproduction from short-term tests

The results of the regression analyses (used to estimate the predictability of lifetime reproduction from shorter-term reproduction data) for the first and second sets of tests are sum-

Table 3. Results of regression analyses for short-term (7-d) reproduction versus lifetime reproduction of *Ceriodaphnia dubia* in full life-cycle tests

Water source	Treatment	Intercept (±SE)	Slope (±SE)	Adjusted R^2	p
Control water	Not filtered, food added	41.39 ± 24.71	3.20 ± 0.97	0.168	0.001
McCoy Branch		−2.69 ± 16.01	3.93 ± 0.55	0.507	<0.001
Lake Reality outfall		79.53 ± 27.89	1.11 ± 0.87	0.013	0.209
Lake Reality outfall	Filtered, no food added	ND[a]	ND	ND	ND
Lake Reality outfall	Filtered, food added	3.96 ± 5.37	2.08 ± 0.24	0.608	<0.001
Lake Reality outfall	Not filtered, no food added	14.52 ± 1.41	−7.02 ± 7.06	−0.0002	0.325
Lake Reality outfall	Not filtered, food added	31.34 ± 23.10	2.00 ± 0.83	0.090	0.019

[a] No reproduction occurred in this treatment combination.

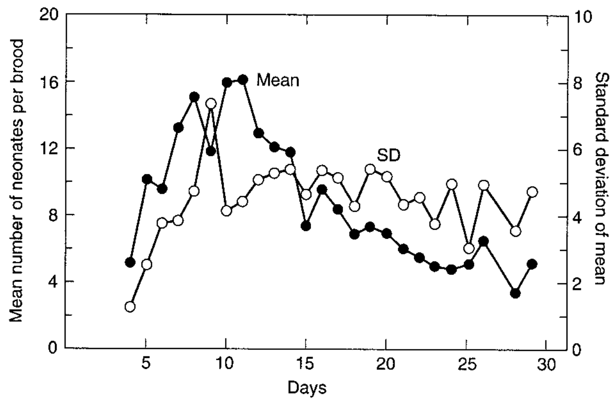

Fig. 3. Mean brood sizes of *Ceriodaphnia dubia* and standard deviation of mean brood sizes during the first set of tests. Data for water from three sources (McCoy Branch, Lake Reality outfall, and control) are combined because of similarities in *C. dubia* survival and reproduction. Means are computed only for days when 10 or more *C. dubia* reproduced (three water types combined). The use of the 10 or more criterion was arbitrary but represents 20% or more of each original "population." Thus, data shown in this figure are generalized to emphasize population-level trends.

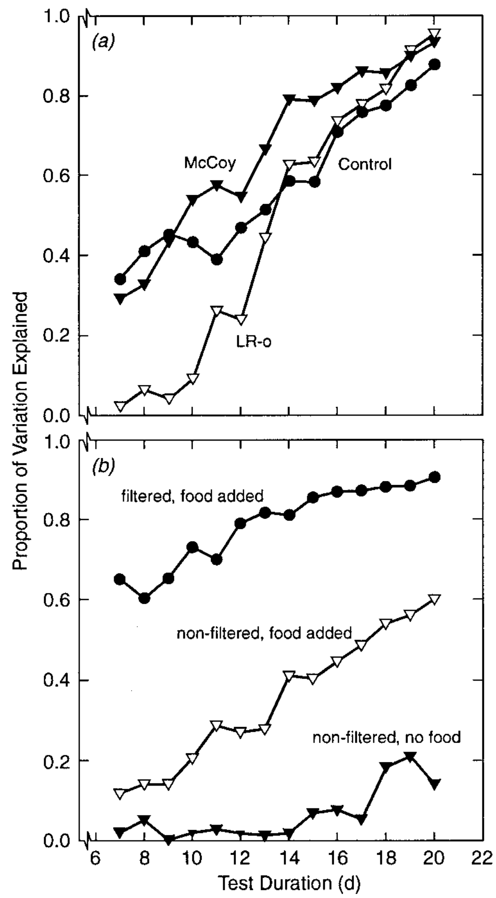

Fig. 5. Proportion of variation in *Ceriodaphnia dubia* lifetime reproduction explained (R^2, from regressions) versus duration of testing in the first (**a**) and second (**b**) set of life-cycle tests. LR-o = Lake Realty outfall.

marized in Table 3 and Figures 5a and 5b, respectively. In the first set of tests, the proportion of variation in lifetime reproduction explained by short-term reproduction data (i.e., R^2) tended to increase as test duration increased, as one would expect, for water from all three sites (Fig. 5a). However, the R^2 for short-term versus lifetime reproduction data for *Ceriodaphnia* in LR-o water was initially much lower than the R^2 values for *Ceriodaphnia* in the other two types of water, and for this water, R^2 values continued to lag behind values from the other two types of water until days 13 to 14.

In the second set of tests, R^2 values for regressions of short-term reproduction data on lifetime reproduction data from *Ceriodaphnia* were strongly affected by the filtering and food-addition treatments (Fig. 5b). The value R^2 for the regressions was high ($>$0.60) for *Ceriodaphnia* in filtered LR-o water (with food added) as early as day 7 and increased to about 0.9 by day 20. In contrast, R^2 values for *Ceriodaphnia* in nonfiltered water with no food added remained low throughout the test.

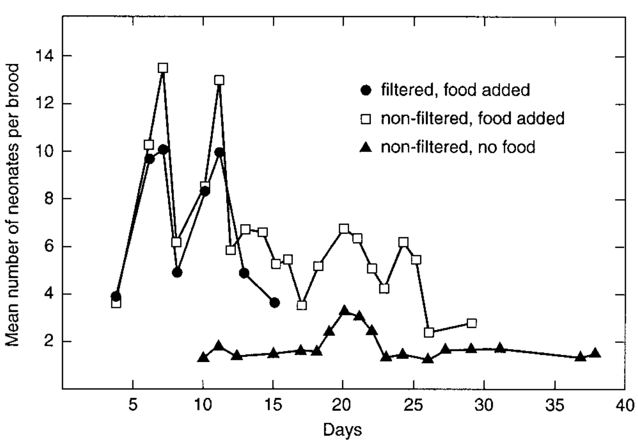

Fig. 4. Mean brood sizes of *Ceriodaphnia dubia* in Lake Reality outfall water, through time, in the second set of tests. For each treatment, means are computed only for days when 10 or more *C. dubia* reproduced. The use of the 10 or more criterion was arbitrary but represents 20% or more of each original "population." Thus, data shown in this figure are generalized to emphasize population-level trends.

DISCUSSION

Test of exposure duration hypothesis

Upper East Fork Poplar Creek and McCoy Branch were historically contaminated with various pollutants, some with bioaccumulation potential (e.g., mercury and PCBs). Although these contaminants still occur in these streams at elevated concentrations in sediments, fish, and periphyton [18,19,21], water samples from LR-o and McCoy Branch have shown little or no evidence of toxicity to *Ceriodaphnia* in 7-d tests despite much testing [7,18]. Thus, we hypothesized that water from these two sites might reveal evidence of chronic toxicity to *Ceriodaphnia* by extending test duration. This hypothesis was not supported by data from either set of full life-cycle tests.

In the first set of tests, we found little difference in survival or reproduction of *Ceriodaphnia* in McCoy Branch or LR-o water compared with control water (Figs. 1a and 2a and Table 2). Additionally, although the daily proportion of *C. dubia* reproducing in McCoy Branch water and LR-o water was lower than that of daphnids in control water after about day 15 (Fig. 2a), *C. dubia* in water from LR-o and McCoy Branch demonstrated some degree of reproductive compensation, notably in terms of the mean number of offspring per brood (Table 2).

In the second set of tests, *Ceriodaphnia* obtaining all of their nutrition from naturally occurring particulate matter in

LR-o water (nonfiltered water, no food added) had, on average, long life spans (Fig. 1b) and sustained reproduction for a long period of time: more than 20% of the animals were still reproducing on day 38 (Fig. 2b). This outcome would not be expected if exposure duration alone accounted for the apparent lack of toxicity based on the results of 7-d tests. For these reasons, we conclude that the apparent lack of toxicity in water from McCoy Branch and LR-o, as determined from numerous 7-d tests with *C. dubia,* probably is not the result of an insufficiently long exposure.

Significance of water quality versus food-related parameters to Ceriodaphnia

In the first set of tests, differences in *C. dubia* longevity and reproduction among the three waters were small, whereas means for conductivity, alkalinity, and hardness differed by factors of 1.7 to 2.5 (Table 1). Mean pH values for the three waters ranged from 8.00 to 8.41, levels well below those found to reduce survival of *C. reticulata* [10]. The small differences in *C. dubia* survival and reproduction among water types in the first set of tests, the large effect of the filtering and food-addition treatments on *C. dubia* survival and reproduction in the second set of experiments, the widespread geographic distribution of *Ceriodaphnia,* and the results of other studies collectively lead us to suggest that in standardized test situations, *Ceriodaphnia* may be more sensitive to ''food issues'' than to ''water issues'' within reasonably broad ranges of water quality conditions.

Daphnids can derive energy from organic matter in various forms. *Ceriodaphnia dubia,* for example, can survive and reproduce in swamp water that is relatively rich in bacteria and low in phytoplankton [22]. Daphnids can even use dissolved organic matter as a food resource, by an adsorptive mechanism involving clay mineral sediments [23–25]. Numerous studies also show that both the types and quantities of algae available as food can influence daphnid growth and reproduction [26–31]. Thus, it is not surprising that we found strong effects of naturally occurring particulate matter, exogenous to that added as a standard food, on the reproductive pattern of *C. dubia* in the second set of tests (Table 2). These considerations also suggest that some *C. dubia* control tests that fail because of unacceptably low reproduction may occur as a result of food problems.

Extrapolation of results from short-term tests to long-term outcomes

Life-span reproduction of *C. dubia* was poorly predicted by reproduction data from the first 7 d of testing in all three types of water in the first set of tests (Fig. 5a) and in two of the three treatment combinations in the second set of tests (Fig. 5b and Table 3). For five of the six test situations, more than 13 d of testing was needed to explain 60% or more of the variation in lifetime reproduction. This was the case even for control water, which did not vary chemically through time. The pattern of change in R^2 with increase in the duration of testing also differed among treatments, notably in the second set of tests (Fig. 5b). Furthermore, even 25 offspring per *C. dubia* female during a 7-day test period, a level of fecundity that appears to be generally achievable by those that are experienced in conducting minichronic tests with this species, is only 20 to 25% of the mean number of offspring that can be produced in a full life-cycle test (Table 2). Collectively, these findings suggest that it is probably not possible to accurately predict life-span production of *C. dubia* from the results of 7-d tests.

Mean longevity of *C. dubia* in nonfiltered LR-o water (food added) in the first test period was similar to that in the second test period (25.6 vs 24.3 d), but the the total number of offspring per female in the second test period was only about 75% as great as in the first test period (Table 2). The difference in mean reproduction between the two test periods was due to fewer neonates per brood, on average, in the second test: the greater number of broods per female, during test period two, was not enough to fully compensate for the smaller number of neonates per brood (Table 2). The test-to-test difference in mean total number of offspring per female, given the same ambient water source, could be due to a number of things, including differences in physicochemical water quality factors (note that water quality parameters differed slightly between the two test periods; Table 1), seasonal changes in the quality or quantity of algae or detritus in Lake Reality, or differences in physiological status of *C. dubia* neonates used to start the two tests. This level of variation is typical in ambient testing with *C. dubia* when using 7-d test procedures, even when only reference stream water samples are being assessed [8].

We do not suggest that poor prediction of longer-term reproduction from the results of shorter-term (e.g., 7-d) tests with *C. dubia* invalidates the use of 7-d test procedures for estimating the biological quality of effluents or receiving waters for two reasons. First, the ecological life span of *Ceriodaphnia* is likely to be far shorter than its laboratory life span because of the presence of diverse invertebrate and vertebrate predators in most natural habitats [32,33]. Second, offspring produced earlier in a daphnid's life are ecologically much more valuable than those produced later [34]. However, our results do suggest that the results of *C. dubia* 7-d ambient tests should be considered carefully in the context of testing objectives, with considerable understanding of the potentially important influences of test methodology and the framework used for statistical assessment. Statistically, for example, the results of *C. dubia* tests might be interpreted more accurately by giving greater consideration to power and minimum significant difference [35], particularly after issues associated with the use of the no-observed-effect concentration as a test endpoint have been resolved [36,37]. The results of our study also support the idea that greater standardization and attention to food used in *C. dubia* testing might decrease intra- and interlaboratory variability [38]. This issue would be particularly important if neonate production by *C. dubia* in short-term tests becomes a preferred endpoint for regulatory compliance.

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REFERENCES

1. Naddy RB, La Point TW, Klaine SJ. 1995. Toxicity of arsenic, molybdenum and selenium combinations to *Ceriodaphnia dubia. Environ Toxicol Chem* 14:329–336.
2. Peters GT, Burton DT, Paulson RL, Turley SD. 1991. The acute and chronic toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine

(RDX) to three freshwater invertebrates. *Environ Toxicol Chem* 10:1073–1081.
3. Weber CI, et al. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2nd ed. EPA/600/4-89/001. U.S. Environmental Protection Agency, Cincinnati, OH.
4. Ong SK, DeGraeve GM, Silva-Wilkinson RA, McCabe JM, Smith WL. 1996. Toxicity and bioconcentration potential of adsorbable organic halides from bleached laundering in municipal wastewater. *Environ Toxicol Chem* 15:138–143.
5. Stewart AJ, Kszos LA, Harvey BC, Wicker LF, Haynes GJ, Bailey RD. 1990. Ambient toxicity dynamics: Assessments using *Ceriodaphnia dubia* and fathead minnow (*Pimephales promelas*) larvae in short-term tests. *Environ Toxicol Chem* 9:367–379.
6. Nimmo DR, Dodson MH, Davies PH, Greene JC, Kerr MA. 1990. Three studies using *Ceriodaphnia* to detect nonpoint sources of metals from mine drainage. *J Water Pollut Control Fed* 62:7–15.
7. Stewart AJ, Hill WR, Ham KD, Christensen SW, Beauchamp JJ. 1996. Chlorine dynamics and ambient toxicity in receiving streams. *Ecol Appl* 6:458–471.
8. Stewart AJ. 1996. Ambient bioassays for assessing water-quality conditions in receiving streams. *Ecotoxicology* 5:377–393.
9. Mount DI, Norberg TJ. 1984. A seven-day life cycle cladoceran toxicity test. *Environ Toxicol Chem* 3:425–434.
10. O'Brien WJ, DeNoyelles F Jr. 1972. Photosynthetically elevated pH as a factor in zooplankton mortality in nutrient enriched ponds. *Ecology* 53:605–614.
11. Kszos LA, Stewart AJ, Taylor PA. 1992. An evaluation of nickel toxicity to *Ceriodaphnia dubia* in a contaminated stream and in laboratory tests. *Environ Toxicol Chem* 11:1001–1012.
12. Kszos LA, Stewart AJ. 1992. Effort-allocation analysis of the seven-day fathead minnow (*Pimephales promelas*) and *Ceriodaphnia dubia* toxicity tests. *Environ Toxicol Chem* 10:67–72.
13. Griest WH, Tyndall RL, Stewart AJ, Caton JE, Vass AA, Ho CH, Caldwell WM. 1995. Chemical characterization and toxicological testing of windrow composts from explosives-contaminated sediments. *Environ Toxicol Chem* 14:51–59.
14. Stewart AJ, Haynes GJ, Martinez MI. 1992. Fate and biological effects of contaminated vegetation in a Tennessee stream. *Environ Toxicol Chem* 11:653–664.
15. Adams SM, Ham KD, Beauchamp JJ. 1994. Application of canonical variate analysis in the evaluation and presentation of multivariate biological response data. *Environ Toxicol Chem* 13:1673–1683.
16. Stewart AJ, Loar JM. 1994. Spatial and temporal variation in biomonitoring data. In Loeb SL, Spacie A, eds, *Biological Monitoring of Aquatic Systems.* Lewis, Boca Raton, FL, USA, pp 91–124.
17. Lotts JW Jr, Stewart AJ. 1995. Minnows can acclimate to total residual chlorine. *Environ Toxicol Chem* 14:1365–1374.
18. Ryon MG. 1992. Ecological effects of contaminants in McCoy Branch, 1989–1990. ORNL/TM-11926. Oak Ridge National Laboratory, Oak Ridge, TN, USA.
19. U.S. Department of Energy. 1994. Remedial investigation report for Chestnut Ridge Operable Unit 2 (filled coal ash pond/upper McCoy Branch) at the Oak Ridge Y-12 Plant, Oak Ridge. DOE/OR/02-1238 DO. Oak Ridge, TN.
20. SAS Institute. 1985. *SAS® User's Guide: Statistics, Version 5 Edition.* Cary, NC, USA.
21. Hill WR, Stewart AJ, Napolitano GE. 1996. Mercury speciation and bioaccumulation in lotic primary producers and primary consumers. *Can J Fish Aquat Sci* 53:812–819.
22. Anderson DH, Benke AC. 1994. Growth and reproduction of the cladoceran *Ceriodaphnia dubia* from a forested floodplain swamp. *Limnol Oceanogr* 39:1517–1527.
23. Arruda JA, Marzolf GR, Faulk RT. 1983. The role of suspended sediments in the nutrition of zooplankton in turbid reservoirs. *Ecology* 64:1225–1235.
24. Kirk KL. 1991. Inorganic particles alter competition in grazing plankton: The role of selective feeding. *Ecology* 72:915–923.
25. Kirk KL, Gilbert JJ. 1990. Suspended clay and the population dynamics of planktonic rotifers and cladocerans. *Ecology* 71:1741–1755.
26. Lynch M. 1992. The life history consequences of resource depression in *Ceriodaphnia quadrangula* and *Daphnia ambigua. Ecology* 73:1620–1629.
27. Infante A, Litt AH. 1985. Differences between two species of *Daphnia* in the use of 10 species of algae in Lake Washington. *Limnol Oceanogr* 30:1053–1059.
28. Richmam S, Dodson SI. 1983. The effect of food quality on feeding and respiration of *Daphnia* and *Diaptomus. Limnol Oceanogr* 28:948–956.
29. Wylie JL, Currie DJ. 1991. The relative importance of bacteria and algae as food sources for crustacean zooplankton. *Limnol Oceanogr* 36:708–728.
30. Sterner RW, Hagemeier DD, Smith WL, Smith RF. 1993. Phytoplankton nutrient limitation and food quality for *Daphnia. Limnol Oceanogr* 38:857–871.
31. Lampert W. 1987. Feeding and nutrition in *Daphnia. Mem Ist Ital Idrobiol Dott Marco Marchi* 45:143–192.
32. Gliwicz ZM, Umana G. 1994. Cladoceran body size and vulnerability to copepod predation. *Limnol Oceanogr* 39:419–424.
33. Tessier AJ, Young A, Liebold M. 1992. Population dynamics and body-size selection in *Daphnia. Limnol Oceanogr* 37:1–13.
34. Threlkeld ST. 1987. *Daphnia* population fluctuations: Patterns and mechanisms. *Mem Ist Ital Idrobiol Dott Marco Marchi* 45:367–388.
35. Denton DL, Norberg-King TJ. 1996. Whole effluent toxicity statistics: A regulatory perspective. In Grothe DE, Dickson KL, Reed-Judkins DK, eds, *Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts.* SETAC, Pensacola, FL, USA, pp 83–102.
36. Laskowski R. 1995. Some good reasons to ban the use of NOEC, LOEC and related concepts in ecotoxicology. *Oikos* 73:140–144.
37. Kooijman SALM. 1996. An alternative for NOEC exists, but the standard model has to be abandoned first. *Oikos* 75:310–316.
38. Ferrari B, Ferard JF. 1996. Effects of nutritional renewal frequency on survival and reproduction of *Ceriodaphnia dubia. Environ Toxicol Chem* 15:765–770.