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Running Head: Life-cycle testing with *Ceriodaphnia dubia*

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Life-cycle testing of receiving waters with *Ceriodaphnia dubia*

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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) see if tests with longer exposure periods would reveal evidence for toxicity that might not be evident from 7-day tests, and (2) determine the relative importance of water quality versus food as factors influencing *C. dubia* reproduction. In the first set of tests, *C. dubia* was reared in diluted mineral water (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, but this water was either filtered or not filtered, and food was either added or not added, prior to testing. *C. dubia* survival and reproduction did not differ much among the three water types in the first set of tests, but these two parameters 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 quite sensitive to food-related factors. Regression analyses showed that the predictability of life-time reproduction by *C. dubia* from the results of 7-day tests was very low ($R^2 < 0.35$) in five of the six experiments. The increase in predictability 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, 7-day tests with *C. dubia* may be used to quantify water-quality problems, but it may not be possible to reliably extrapolate the results of these tests to longer time scales.

Key words: *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 wide-spread 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, 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 can be 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, in cases where laboratory toxicity test results are used to estimate ecological risk. The accuracy or limitations of extrapolative estimates of risk that use the results of *C. dubia* tests might be assessed more clearly by better understanding of the basic biology of these organisms in toxicity testing situations.

In this study we report the results of several full life-cycle tests with *C. dubia*. The tests reported here were conducted determine if longer exposure to water from two streams known to contain contaminants with a tendency to bioaccumulate might unveil adverse physiological effects not detectable using conventional 7-day test procedures.

Materials and Methods

The test methods used in this study were similar to those 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 ten replicates (one daphnid per beaker), we started with 50 replicates (one daphnid per beaker). Second, rather than ending a test seven days after it had been started, we continued daily transfers of the animals (and daily counts of their offspring) until the last animal had died.

Two sets of full life-cycle tests were conducted. The first was initiated on July 27, 1990 and lasted 41 days. In this set of tests, *Ceriodaphnia* neonates < 24 h old 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 impoundment of East Fork Poplar Creek (EFPC) located near the Department of Energy's Oak Ridge Y-12 Plant in eastern Tennessee. EFPC near Lake Reality has been well characterized biologically and toxicologically [7, 14-17]; the sediments in this stream contains various pollutants, including mercury and polychlorinated biphenyls (PCBs). McCoy Branch, a first-order stream located about 5 km southwest of Lake Reality, was historically contaminated with fly-ash. This material 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. Historical contaminants of concern in McCoy Branch stream include arsenic and selenium [18-19].

The second set of tests was initiated on March 9, 1993 and lasted 62 days. In this set of tests, *Ceriodaphnia* neonates < 24 h old were tested in water obtained 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 fed (100 μL of food per beaker per day, given when water was renewed each day) or not fed.

In both sets of tests, food, when supplied, consisted of a standard mixture of yeast, fermented trout chow, cerophyll and algae [3]. In both sets of tests, water samples from the ambient sites (e.g., LR-o or

McCoy Branch) were collected as grab samples three times weekly (Monday, Wednesday and Friday) between 0800 and 1000. The freshly collected grab samples of water were analyzed for pH, conductivity, alkalinity [Environmental Protection Agency (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 offspring produced, on each day, divided by the number of living females on that day. Reproductive synchrony of *Ceriodaphnia* was inspected by plotting the percentage of live adult daphnids that produced any offspring on each day of each test. Mean brood size was computed daily, for each test, by dividing the total number of offspring produced, on any day, by the number of daphnids producing any offspring on that day. 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 explore the effects of the filtering versus non-filtering treatment, and the food versus 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. Regression analysis was applied to data from both sets of life-cycle tests to determine if the number of offspring produced per female by the end of the seventh day of a test, for each treatment combination, could be used to reliably predict life-time reproduction. These regression analyses used data only from animals that lived at least 15 days.

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 events occurred during the first set of tests, 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 to the first set of tests (Table 1).

Table

Ceriodaphnia *survival*

In the first set of tests, survival patterns of *Ceriodaphnia* in the three water types were similar (Fig. 1). 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 d, 27.0 d and 26.4 d respectively; the maximum longevity of *C. dubia* in any of the waters was 41 d. The daily maximum difference in *Ceriodaphnia* survival among the three water types increased gradually to a maximum of about 10 days (between days 17 and 24) before declining (Fig. 1).

Fig. 1

In the second set of tests, *C. dubia* survival patterns differed markedly among the four treatments (Fig. 2). Maximum longevity was as short as 14 day for non-fed animals in filtered LR-o water, and as

Fig. 2

long as 62 days for non-fed daphnids in non-filtered LR-o water. The length of time needed for 50% of the daphnids to die ranged from 7.2 days for non-fed animals in filtered LR-o water, to 32 days for non-fed animals in non-filtered LR-o water (Fig. 2).

Ceriodaphnia *reproduction synchrony*

In the first set of tests, mean daily reproduction of *C. dubia* occurred predominately as an extended series of regularly spaced pulses (Fig. 3). The pulses generally were 2 days in duration, and were separated from one another by 1-day 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 15-20 days of the tests. Then, 80% to 100% of the daphnids released offspring on days 4-5, 7-8, 10-11, 13-14, etc. (Fig. 3). However, even on the "low-production" days that occurred between reproductive pulses, 10% to 30% of the *C. dubia* released offspring.

Fig. 3

In the second set of tests, the reproductive patterns of *C. dubia* were less regular than those noted in the first set of tests, and the degree of reproductive synchrony was affected by the filtration and food-addition treatments (Fig. 4). In non-filtered 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 the non-filtered LR-o water with food added, the proportion of the daphnids that reproduced each day was generally greater than the proportion of daphnids reproducing each day in the filtered LR-o water to which food was added, particularly after day 20 (Fig. 4).

Fig. 4

Ceriodaphnia *brood-sizes*

The mean total number of offspring per daphnid, the mean largest brood per daphnid, the 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, 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 and could have masked significant day-to-day differences in brood size. To explore this possibility we plotted mean brood size and standard deviation of the mean brood size versus day for *Ceriodaphnia*, pooling data for the three types of water. For this set of tests, pooling was justified on the basis of similarity in survival curves (Fig. 1) and total reproduction (Table 2). To ensure that the analysis was robust, only data from days when 10 or more daphnids reproduced were included. Mean brood size increased more or less steadily from about 5 offspring/brood (first-brood data, day 4) to a maximum of about 16 offspring/brood (day 11), then gradually declined from day 11 to day 24 (Fig. 5). A small increase in mean brood size occurred during days 25-27 (Fig. 5), when 44% to 60% of the daphnids still survived (Fig. 1). Except for one unusually high value on day 9, standard deviation of mean brood size increased steadily for the first five days of reproduction, from 1.1 to 4.8; it then appeared to plateau, with values ranging from about 4 to 5.5.

In the second set of tests, mean brood size (for days when 10 or more daphnids reproduced) was consistently low for the non-filtered, no-food-added treatment, and higher and more variable in the filtered and non-filtered water to which food was added (Fig. 6). 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 standard deviations for mean brood size (by day, computed as described above) for the three treatments were 0.4 to 1.6 (non-filtered, no-food-added treatment), 1.1 to 4.7 (non-filtered, food added treatment), and 1.5 to 4.1 (filtered, food-added treatment).

Life-time 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 versus 11.9), but a larger number of broods (12.4 versus 9.7), compared to *Ceriodaphnia* reared in LR-o water (Table 2). ANOVA 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 non-filtered food-augmented LR-o water produced only about 75% as many offspring as *Ceriodaphnia* in the non-filtered LR-o food-augmented water that was used in the first set of tests (86.1 versus 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 *Ceriodaphnia*'s mean lifespan from 6.6 d to 31.6 days. 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). ANOVA 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 lifespan (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 life-time reproduction from short-term tests

The results of the regression analyses (used to estimate the predictability of life-time reproduction from shorter-term reproduction data) for the first and second sets of full life-cycle tests are summarized in Figs. 7 and 8 respectively. In the first set of tests, the proportion of variation in life-time reproduction explained by short-term reproduction data (i.e., R^2) tended to increase as test duration increased, for water from all three sites (Fig. 7). However, the R^2 for short-term versus life-time reproduction data for *Ceriodaphnia* in McCoy Branch 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 values from the other two types of water until days 13-14. Fig. 7

In the second set of tests, R^2 values for regressions of short-term reproduction data on life-time reproduction data from *Ceriodaphnia* were strongly affected by the filtering and food-addition treatments (Fig. 8). R^2 for the regressions was high ($\geq$ 0.60) for *Ceriodaphnia* in filtered (+ food) LR-o water as early as day 7, and increased to about 0.9 by day 20. In contrast, R^2 values for *Ceriodaphnia* in non-filtered water with no food added remained low throughout the test (Fig. 8). Fig. 8

Discussion

Exposure-duration hypothesis

Upper East Fork Poplar Creek and McCoy Branch were historically contaminated with various pollutants, some with bioaccumulation potential (e.g., mercury, PCBs). Although some of 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 for toxicity to

Ceriodaphnia in 7-day tests, despite much testing **[7, 18]**. Thus, we hypothesized that water from these two sites might reveal evidence for chronic toxicity to *Ceriodaphnia* by extending test duration. This hypothesis was not supported by either set of full life-cycle tests.

In the first set of tests, we found little difference in survival or reproductive patterns of *Ceriodaphnia* in McCoy Branch or LR-o water, compared to control water (Figs. 1, 3; Table 2). Additionally, although the daily proportion of daphnids reproducing in McCoy Branch water and LR-o water was lower than that of daphnids in control water after about day 15 (Fig. 3), *Ceriodaphnia* tested 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 full life-cycle tests, *Ceriodaphnia* that were required to obtain all of their nutrition from naturally occurring particulate matter in LR-o water (non-filtered water, no food added) had long life spans, on average (Fig. 2) and sustained reproduction for a long period of time: more than 20% of the animals were still reproducing on day 38 (Fig. 4). This outcome would not be expected if exposure duration alone accounted for the apparent lack of toxicity based on the results of 7-day 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-day tests with *C. dubia*, is not the result of an insufficiently long test duration.

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, while 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 *Ceriodaphnia 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 wide-spread 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.

Algae probably constitute the largest component of the diet of most species of cladocerans in natural habitats, but daphnids also can derive energy from organic matter in various other forms. *C. 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] (but see also [24-25]). Finally, numerous studies 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 observed strong effects of naturally occurring particulate matter, exogenous to that added as a standard food, on fecundity patterns of *C. dubia* in the second set of life-cycle tests (Table 2). These considerations also suggest that *C. dubia* toxicity control-test failures, in which reproduction is unacceptably low, may be more commonly linked to food problems than to reference-water problems.

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

Life-span reproduction of *C. dubia* was poorly predicted from the results of 7-d tests in all three types of water in the first set of life-cycle tests (Fig. 8), and in two of the three treatment combinations in the second set of life-cycle tests (Fig. 8). For five of the six test situations, more than 14 d of testing was needed to explain even half of the variation in life-time reproduction. The pattern of change in R^2 with increase in the duration of testing also differed among the imposed treatments; this was especially evident in the second set of life-cycle tests (Fig. 8). Furthermore, 25 offspring per *C. dubia* female during a 7-day test -- a level of fecundity that is deemed acceptable and generally achievable by those that routinely conduct tests with this species -- represents only 20 to 25% of the mean number of offspring that can be produced in a full life-cycle test (Table 2). These findings suggest that it is probably not possible to accurately predict life-span production from the results of 7-day tests.

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-day test procedures for estimating the biological quality of effluents or receiving waters, for two reasons. First, *Ceriodaphnia*'s ecological life-span is likely to be far shorter than its laboratory life-span due to 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-day 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 (D. Denton, USEPA Region 9, San Francisco, CA; personal communication), particularly after issues associated with the use of the no-observed-effect concentration (NOEC) as a test endpoint have been resolved [35-36]. The results of our study also support the idea that greater standardization and attention to food used in *C. dubia* testing could decrease intra- and interlaboratory variability (cf. [37]): this issue is particularly important if neonate production by *C. dubia* in short-term tests becomes a preferred endpoint for regulatory compliance.

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Table 1. Water-quality parameters (mean ± SD for two sets of life-cycle tests with *Ceriodaphnia dubia*. The number of observations for parameters in the first and second set of tests was 14 and 12, respectively.

Test set	Water source	pH (SU)	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
1	McCoy Branch	8.00 ± 0.10	343 ± 24	163.2 ± 8.7	186.7 ± 6.8
1	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

Table 2. Mean lifespan and means of various reproductive parameters (± SE) for *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	Lifespan (days)	Total no. of offspring per female	Largest brood	No. of broods	Offspring per brood
Control water	none	23.1	119.6 ± 7.7	17.4 ± 0.5	12.4 ± 0.6[b]	9.8 ± 0.3
McCoy Branch	none	24.3	107.2 ± 6.6	18.1 ± 0.6	10.1 ± 0.5	10.7 ± 0.4
Lake Reality outfall	none	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	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	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.

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

Water source	Treatment	intercept (± SE)	slope (± SE)	adjusted R^2	p
Control water	none	41.39 ± 24.71	3.20 ± 0.97	0.168	0.001
McCoy Branch	none	-2.69 ± 16.01	3.93 ± 0.55	0.507	< 0.001
Lake Reality outfall	none	79.53 ± 27.89	1.11 ± 0.87	0.013	0.209
Lake Reality outfall	filtered, no food	---[a]	---	---	---
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	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.

Figure legends

Figure 1. Survival patterns of *C. dubia* in water from McCoy Branch, Lake Reality outfall (LR-o), and control water, first set of life-cycle tests.

Figure 2. Survival patterns of *C. dubia* in water from Lake Reality outfall, second set of life-cycle tests.

Figure 3. Pattern of reproductive synchrony of *C. dubia* in water from McCoy Branch, Lake Reality outfall (LR-o), and control water, first set of life-cycle tests .

Figure 4. Pattern of reproductive synchrony of *C. dubia* in water from Lake Reality outfall, second set of life-cycle tests.

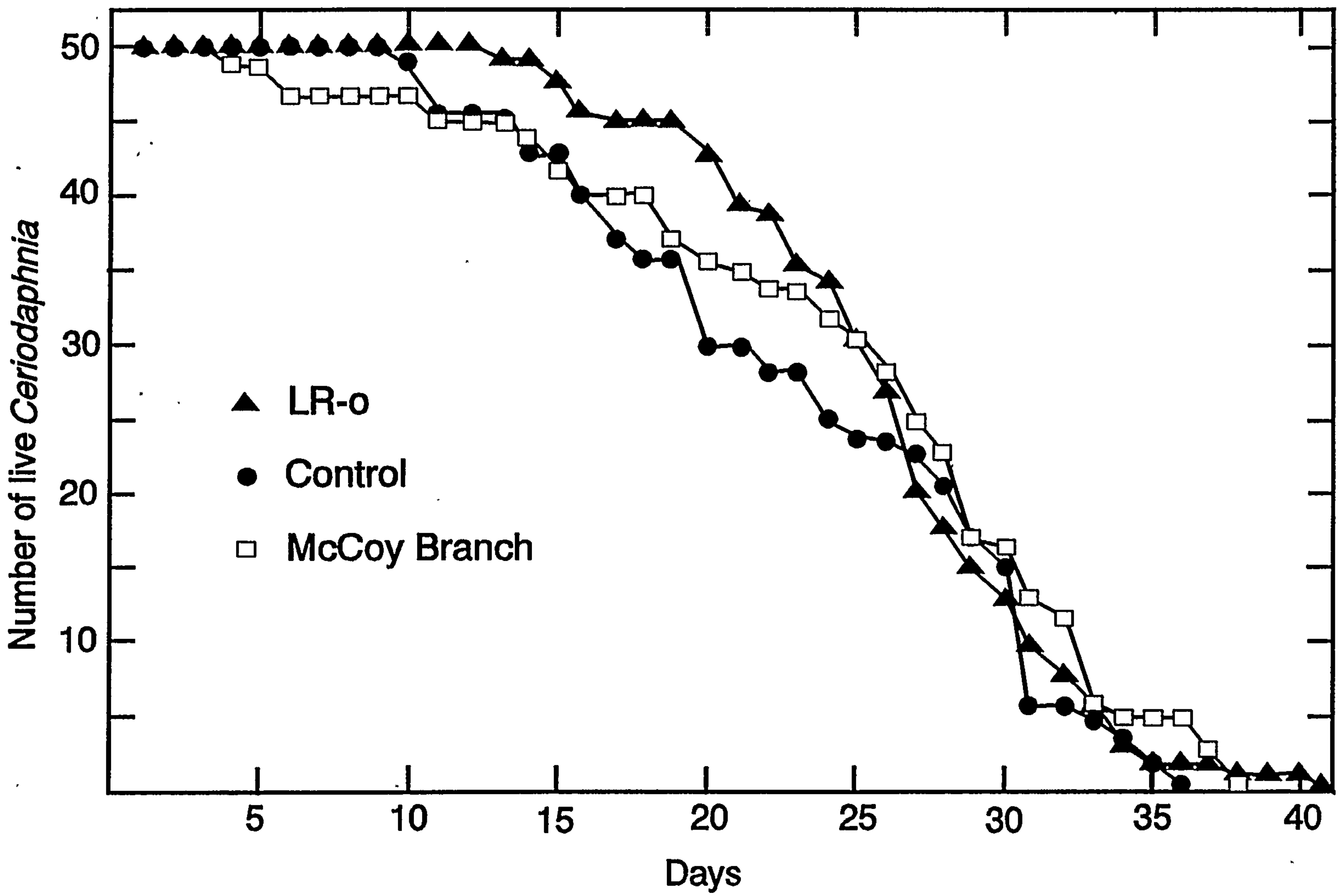
Figure 5. Mean brood sizes of *C. dubia*, and standard deviation of mean brood sizes, during the first set of life-cycle tests. Data for water from three sources (McCoy Branch, Lake Reality outfall, and control) are combined due to similarities in *C. dubia* survival and reproduction.

Figure 6. Mean brood size of *C. dubia* in Lake Reality water, through time, during the second set of life-cycle tests.

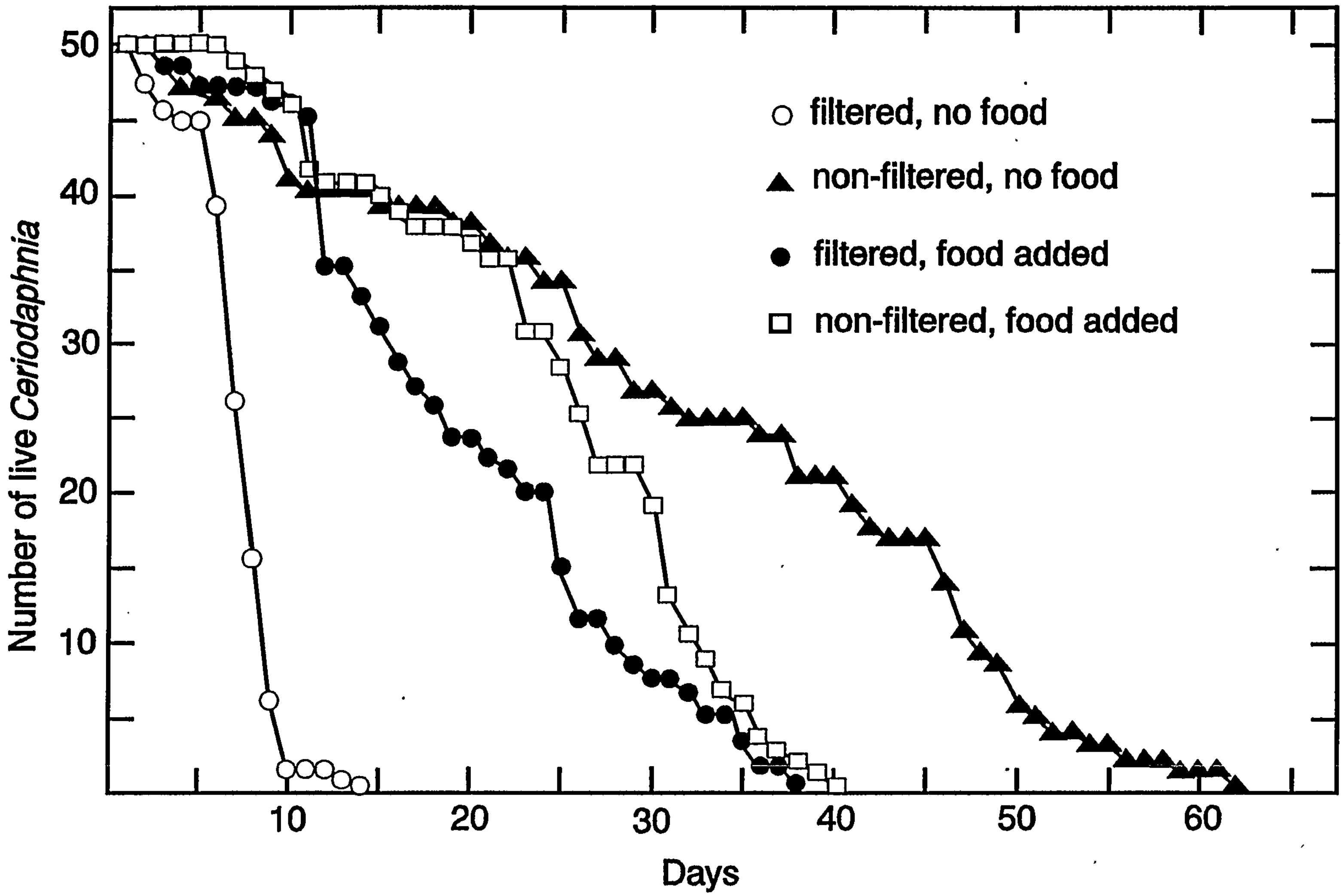
Figure 7. Proportion of variation in *C. dubia* life-time reproduction explained (R^2, from regressions) versus duration of testing, first set of life-cycle tests.

Figure 8. Proportion of variation in *C. dubia* life-time reproduction explained (R^2, from regressions) versus duration of testing, second set of life-cycle tests.

Fig. 1



Number of live Ceriodaphnia
50
40
30
20
10
5
10
15
20
25
30
35
40
Days
LR-o
Control
McCoy Branch



filtered, no food
non-filtered, no food
filtered, food added
non-filtered, food added
Number of live Ceriodaphnia
Days
50
40
30
20
10
10
20
30
40
50
60

Fig. 2

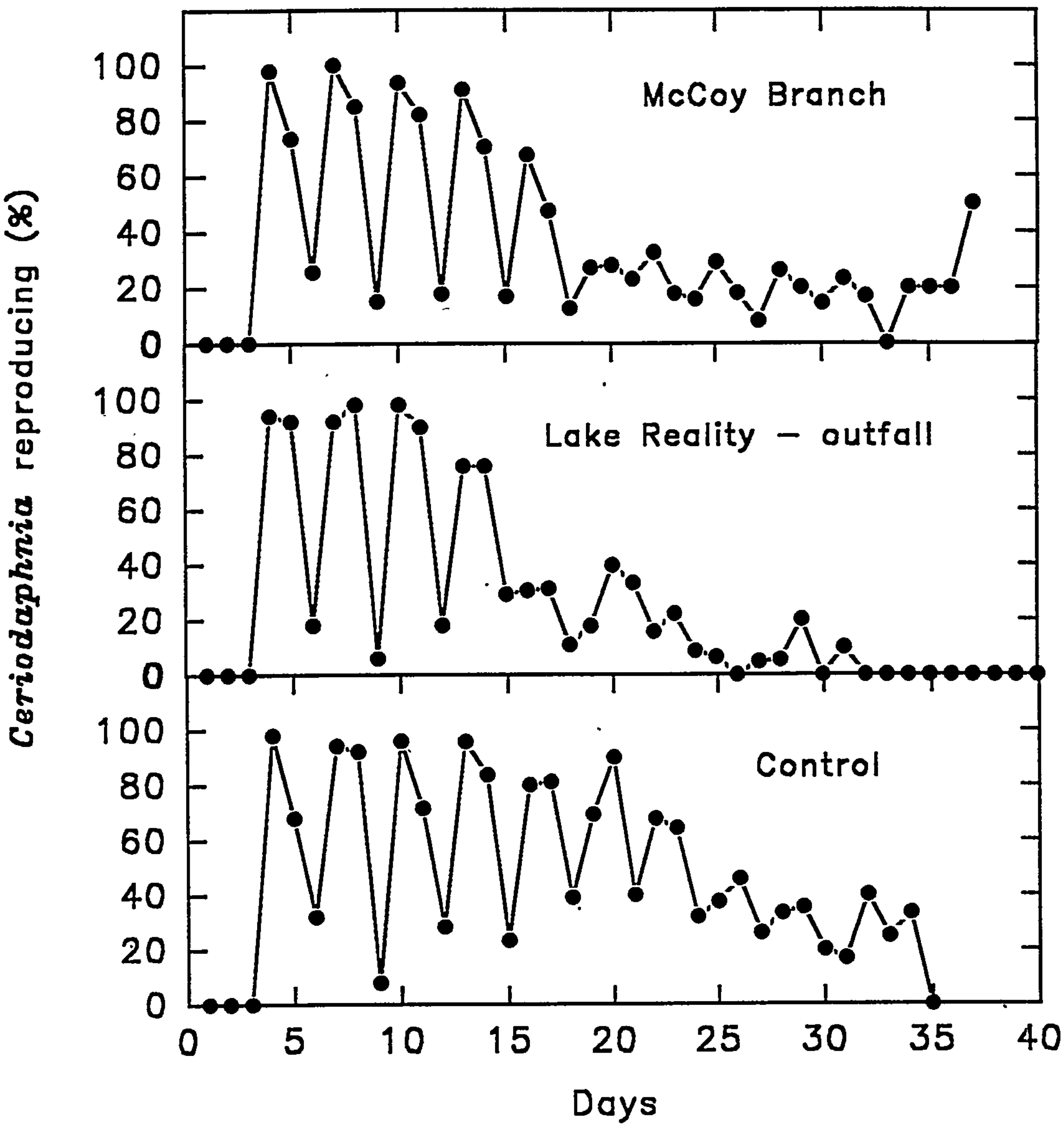
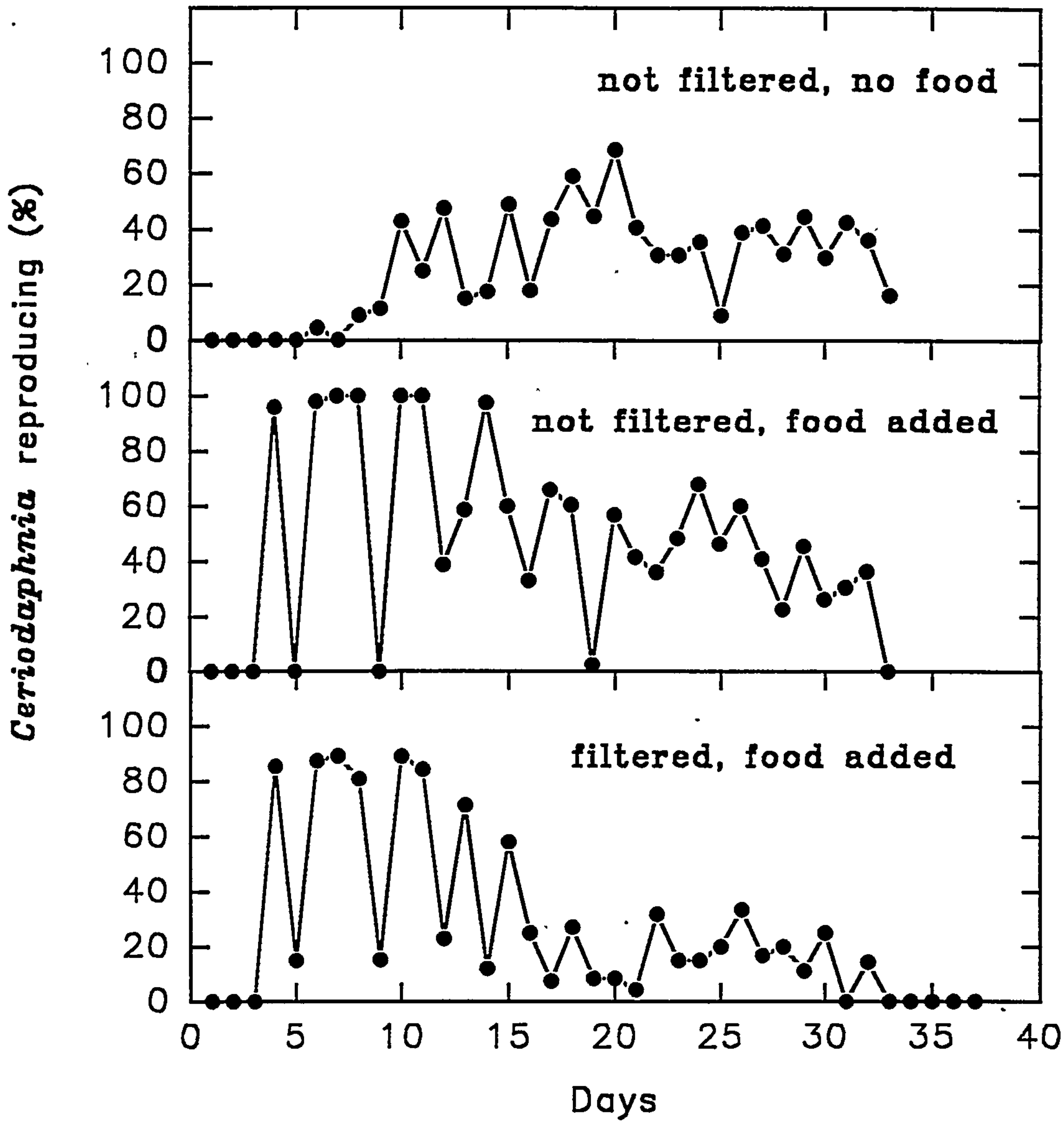
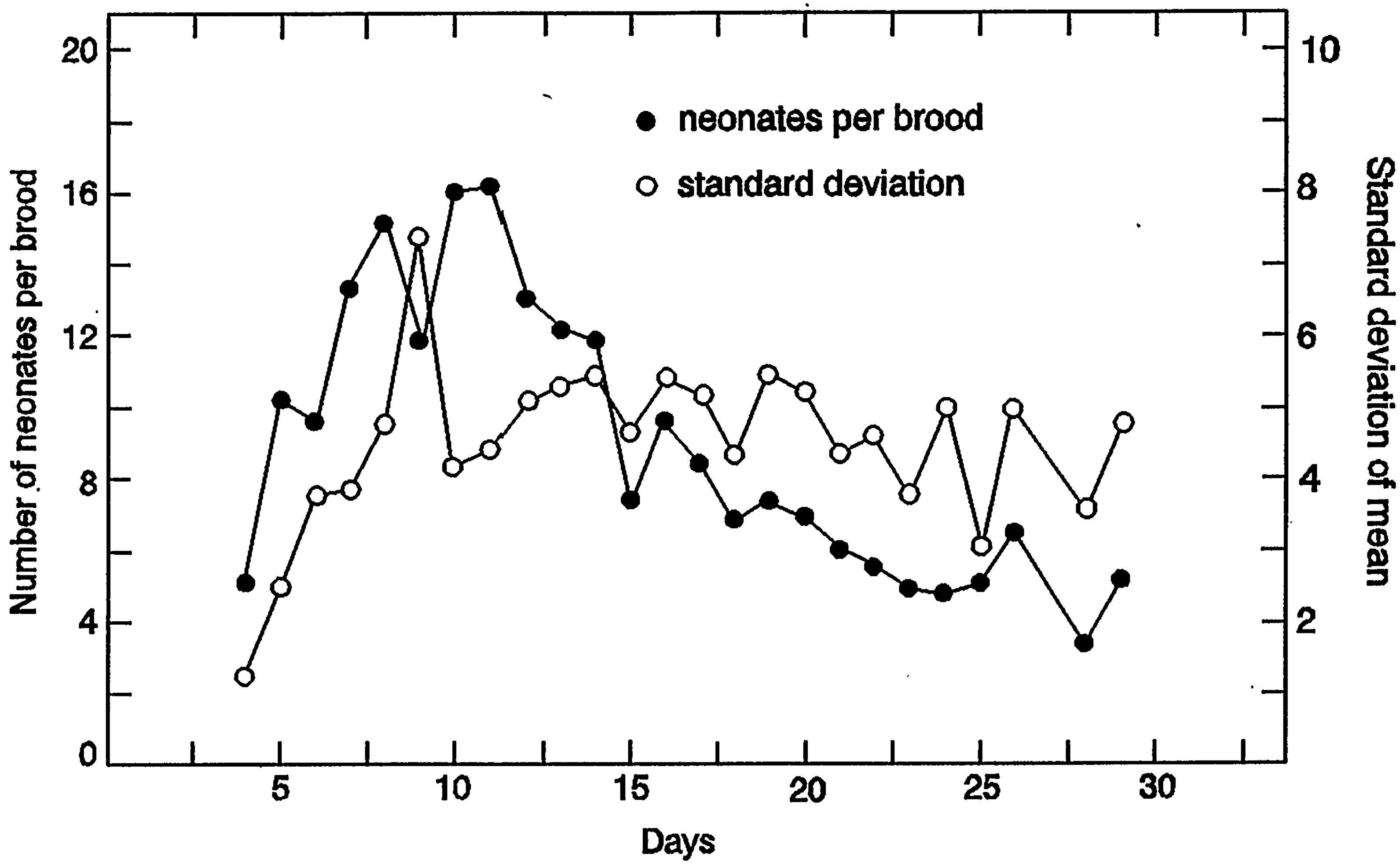

McCoy Branch
Lake Reality – outfall
Control
Ceriodaphnia reproducing (%)
Days
0
5
10
15
20
25
30
35
40
0
20
40
60
80
100

Fig. 3

Fig. 4



not filtered, no food
not filtered, food added
filtered, food added
Ceriodaphnia reproducing (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Days



neonates per brood
standard deviation
Number of neonates per brood
Standard deviation of mean
Days
0
4
8
12
16
20
2
4
6
8
10
5
10
15
20
25
30

Fig. 5

Fig. 6

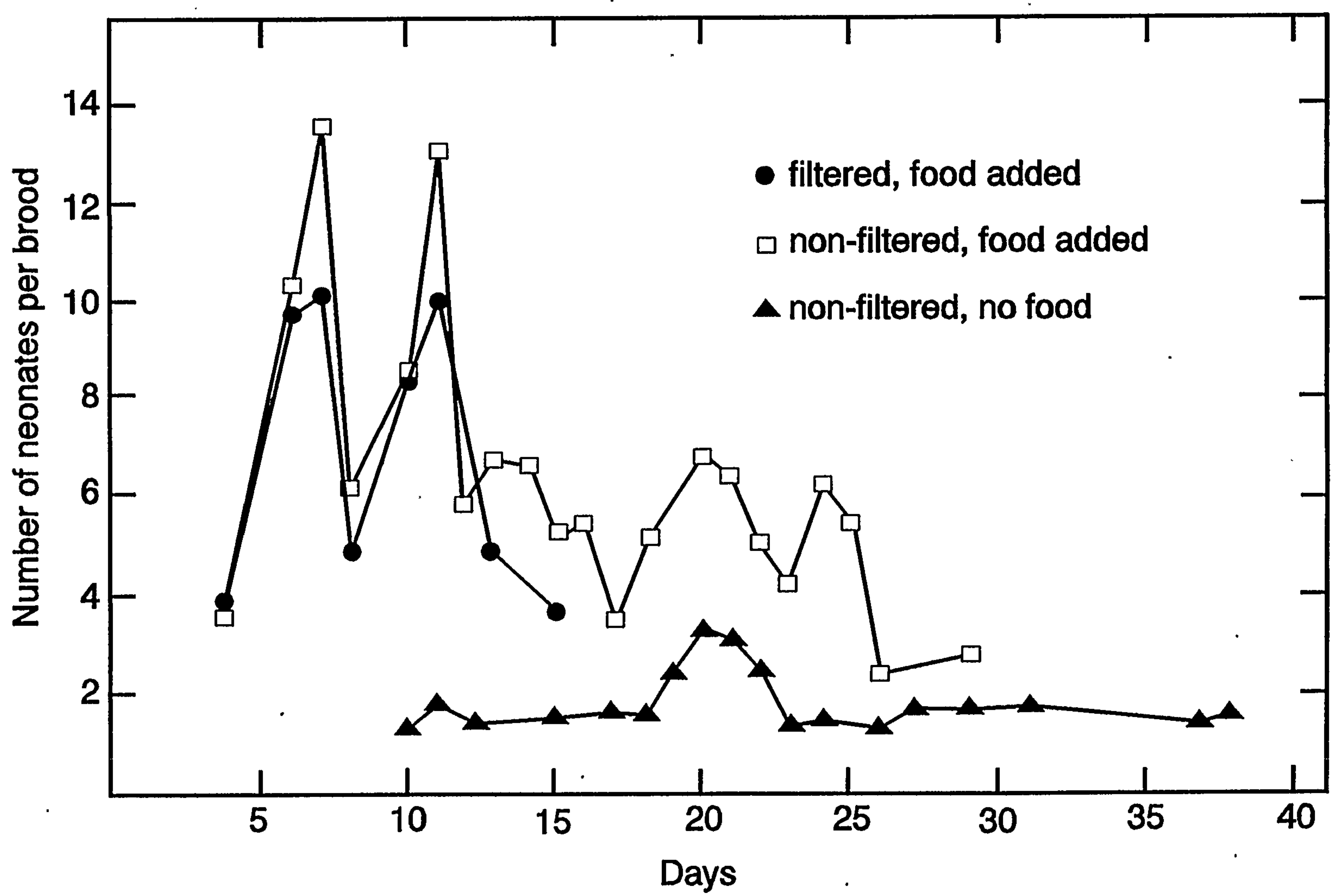
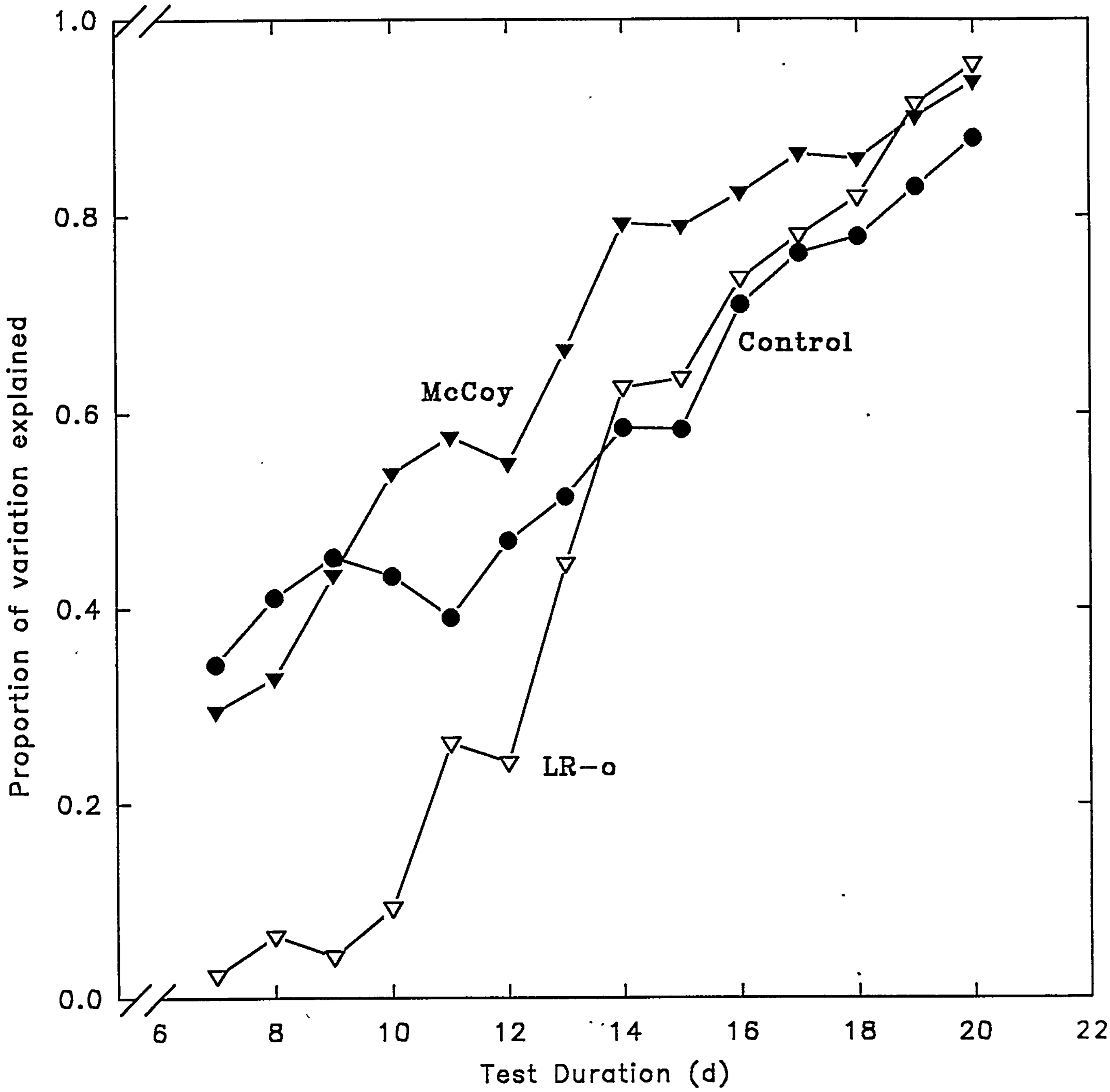
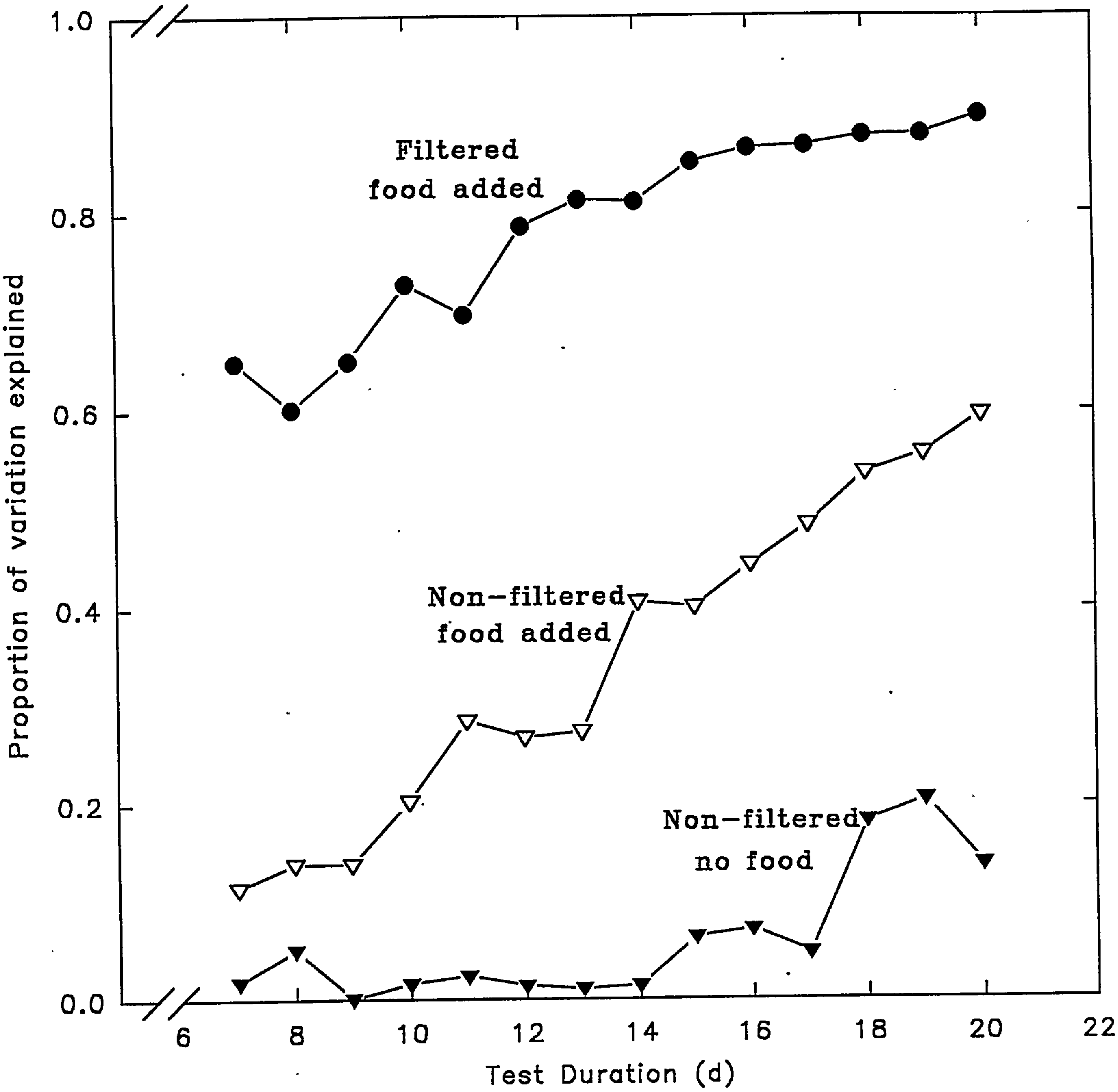

filtered, food added
non-filtered, food added
non-filtered, no food
Number of neonates per brood
14
12
10
8
6
4
2
5
10
15
20
25
30
35
40
Days

Fig. 7



1.0
0.8
0.6
0.4
0.2
0.0
Proportion of variation explained
McCoy
Control
LR-o
6
8
10
12
14
16
18
20
22
Test Duration (d)

Fig. 8


Filtered
food added
Non-filtered
food added
Non-filtered
no food
Proportion of variation explained
Test Duration (d)
1.0
0.8
0.6
0.4
0.2
0.0
6
8
10
12
14
16
18
20
22