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Field Verification of Cadmium Toxicity to Laboratory *Daphnia* Populations[1]

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Although the results of laboratory toxicological experiments with Daphnia have been used to develop water quality criteria for environmental protection purposes, WINNER and FARRELL (1976) point out that extrapolation from laboratory experiments to the natural environment is the crucial problem. STEPHAN and MOUNT (1973) and SPRAGUE (1976) indicate further that the results of laboratory toxicity tests have seldom been verified in the field and that more emphasis on field experiments to verify laboratory predictions is desirable. Unfortunately the results of acute mortality experiments and reproductive impairment tests using Daphnia cannot be verified in the field because mortality and reproduction are not easily distinguished in natural populations. It is possible, however, to determine the effects of toxic stress on both laboratory and natural populations' rates of increase, r — a statistic that integrates the effects on mortality and reproduction and is potentially more useful than those based on either mortality or reproduction alone (MARSHALL, 1962).

The purpose of this paper is to compare the short-term toxicity of cadmium to laboratory populations of D. galeata mendotae with that to natural populations of D. galeata mendotae and total Daphnia in Lake Michigan, as indicated by its effects on the populations' rates of increase.

Materials and Methods

Laboratory populations of D. galeata mendotae were derived from a culture maintained at the Environmental Research Laboratory—Duluth (U. S. Environmental Protection Agency). Four populations were established for each of five levels of added cadmium (0, 5, 10, 15, and 20 μg Cd/l) and were maintained in 500 ml of medium in 600-ml polypropylene beakers. The medium consisted of filtered Lake Michigan water to which 2×10^4 cells/ml of Chlamydomonas

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reinhardi and prescribed amounts of $CdCl_2$ were added and was changed daily Mondays through Fridays (with a triple ration of food on Fridays). The populations were kept in dark incubators at 18.5 ± 0.5°C except for the few minutes required for daily manipulations. Total numbers of individuals in each population were determined at the initiation of the experiment and again after 7 and 14 days.

Natural populations of Daphnia (D. galeata mendotae, D. retrocurva, and D. longiremis) are present in the Lake Michigan zooplankton community. Five in situ experiments were conducted in northern Green Bay, Lake Michigan, off Sister Bay, Wisconsin. In each experiment, two to four opaque polyethylene carboys (8- or 20-liter capacity) for each of five levels of added cadmium were filled with water from the lower portion of the epilimnion (6 to 12 meters). Two to four additional carboys, which were not to be incubated, were filled for determinations of initial numbers of Daphnia. The concentrations of added cadmium were: 0, 50, 100, 150, and 200 µg Cd/l in Experiments One to Three; 0, 25, 50, 75, and 100 µg Cd/l in Experiment Four; and 0, 5, 10, 20, and 40 µg Cd/l in Experiment Five. After the cadmium had been added, the carboys were suspended in the epilimnion for in situ incubation. Water temperature during the five experiments ranged from 17 to 21°C but was essentially constant during each one. After incubation, the zooplankton in each carboy (sample) were removed and preserved in 5 percent formalin. D. galeata mendotae and other species in each sample were enumerated in an open-top, chambered counting cell, using a binocular dissecting microscope. Special care was taken to count only those specimens that showed no signs of decomposition.

Rates of increase, r, for both laboratory and natural populations were calculated from the equation, $r = (\ln \overline{N}_t - \ln \overline{N}_i)/\Delta t$, where $\overline{N}_t$ is the average terminal number of D. galeata mendotae or total Daphnia in populations (samples) at a given cadmium level for a given exposure time, N_i is the average initial number, and Δt is the exposure (incubation) time in days.

Results

The average numbers of D. galeata mendotae in laboratory and natural populations for each added cadmium concentration at the beginning and end of exposure periods of 7—9 days, together with the corresponding values of r, are shown in TABLE 1. The relationships between the rates of increase (r) and added cadmium (µg/l) for laboratory and natural populations of D. galeata mendotae after exposures of 7—9 days are represented by the two linear regression equations and corresponding lines fitted to the data shown

TABLE 1

Mean numbers of Daphnia galeata mendotae in laboratory and natural (in situ) populations at initiation ($\overline{N}_i$) and termination ($\overline{N}_t$) of exposures of 7—9 days to different concentrations of added cadmium, together with calculated rates of increase (r) at each added cadmium concentration.

Added Cd (µg/l)	Laboratory populations			Natural populations		
	$\overline{N}_i$	$\overline{N}_t$	r	$\overline{N}_i$	$\overline{N}_t$	r
0	23 ± 1 [a]	114 ± 9	+0.229	10.7 ± 1.2	7.7 ± 3.7	-0.037
5	20 ± 2	70 ± 5	+0.179	10.7 ± 1.2	5.7 ± 1.8	-0.070
10	24 ± 3	54 ± 10	+0.116	10.7 ± 1.2	4.5 ± 1.5	-0.096
15	22 ± 3	42 ± 5	+0.092	---	---	---
20	23 ± 2	30 ± 2	+0.038	10.7 ± 1.2	2.0 ± 0.0	-0.186
40	---	---	---	10.7 ± 1.2	.25 ± .25	-0.417

(a) Mean ± standard error.

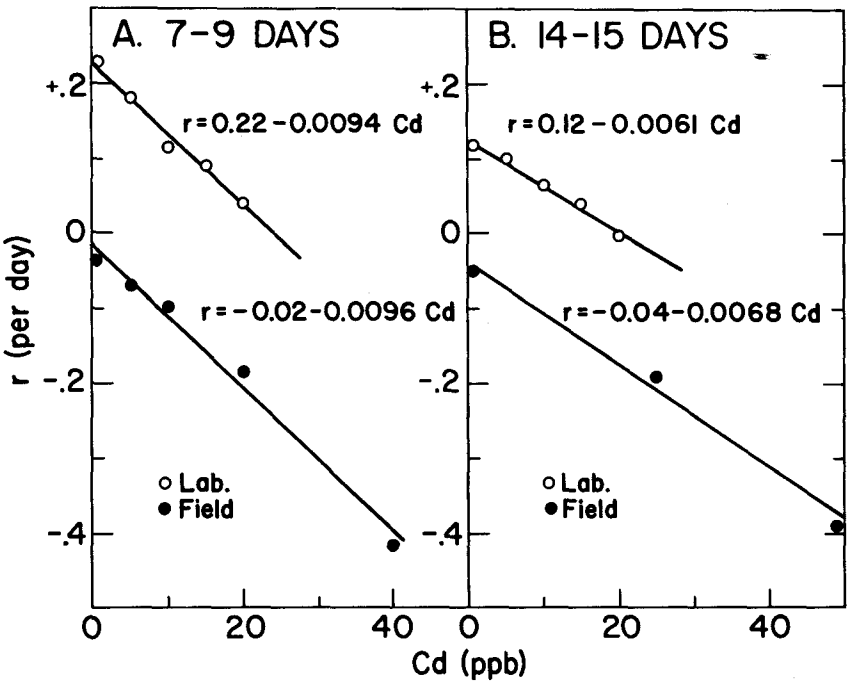

Figure 1. Effects of cadmium on rates of increase of laboratory and field (in situ) populations of Daphnia during exposures of 7—9 days (A) and 14—15 days (B).

in Figure 1 (A). The difference between the slopes, as indicated by a t-test, is not significant ($P > 0.9$). The average numbers of Daphnia (D. galeata mendotae + D. retrocurva + D. longiremis) in laboratory and natural populations for each added cadmium concentration (0—50 μg/l) at the beginning and end of exposure periods of 14—15 days, together with the corresponding values of r, are shown in TABLE 2. The relationships between the rates of increase (r) and added cadmium (μg/l) for laboratory and natural populations of Daphnia after exposures of 14—15 days are represented by the two linear regression equations and corresponding lines fitted to the data shown in Figure 1 (B). Again, the difference between the two slopes is not significant ($P > 0.9$).

The slopes of the regressions for laboratory populations of D. galeata mendotae for exposures of 7 days and 14 days, on the other hand, are significantly different ($P < 0.05$); and those for natural Daphnia populations for exposures of 9 days and 15 days are almost significantly different ($0.1 > P > 0.05$). The relationship between the regression slope and exposure time is further demonstrated by the results of the five in situ experiments. Days

TABLE 2

Mean numbers of Daphnia in laboratory and natural (in situ) populations at initiation ($\overline{N}_i$) and termination ($\overline{N}_t$) of exposures of 14—15 days to different concentrations of added cadmium, together with calculated rates on increase (r) at each added cadmium concentration.

Added Cd (μg/l)	Laboratory populations			Natural populations		
	$\overline{N}_i$	$\overline{N}_t$	r	$\overline{N}_i$	$\overline{N}_t$	r
0	23 ± 1[a]	123 ± 6	+0.120	89 ± 8	42 ± 6	-0.050
5	20 ± 2	80 ± 6	+0.099	---	---	---
10	24 ± 3	61 ± 5	+0.067	---	---	---
15	22 ± 3	39 ± 1	+0.041	---	---	---
20	23 ± 2	22 ± 2	-0.003	---	---	---
25	---	---	---	89 ± 8	5 ± 2	-0.192
50	---	---	---	89 ± 8	.25 ± .25	-0.392

(a) Mean ± standard error.

of exposure (incubation) and linear regression slopes for the relationships between rates of increase (r) and added cadmium (0—50 μg Cd/l) for natural Daphnia (D. galeata mendotae + D. retrocurva + D. longiremis) populations for each in situ experiment are shown in TABLE 3. Note that the absolute value of the regression slope decreases with increasing exposure time. The correlation coefficient (0.893) for the relationship between exposure time and regression slope is significant ($P < 0.05$).

TABLE 3

Days of incubation (exposure time) and linear regression slopes for the relationships between rate of increase (r) and added cadmium (0—50 μg Cd/l) for natural Daphnia populations in five in situ experiments in Green Bay, Lake Michigan.

Experiment	Days	Regression slope
1	4	-0.0199
2	5	-0.0235
3	7	-0.0142
4	15	-0.0068
5	9	-0.0120

Discussion

For the laboratory populations of D. galeata mendotae at least, the slopes of the regressions representing the relationships between r and Cd are a measure of the direct toxicity of cadmium, integrating its effects on mortality and reproduction. This is not necessarily true for the natural populations. However, the observed effects on the different species' values of r probably were due to direct cadmium toxicity because the effect in all cases was a reduction of r. Furthermore the exposure times were probably too short for indirect (secondary) effects to become significant, and the effects of enclosure per se were minor (MARSHALL and VAN REKEN, 1977). Since the regression slopes for the relationships between r and Cd for laboratory and natural populations of D. galeata mendotae are not significantly different, I conclude that the laboratory and natural populations do not differ significantly in their sensitivity to cadmium.

It is interesting to note that the toxicity of cadmium to the laboratory and natural populations, as indicated by the slopes of the regressions of r and Cd, was the same even though the r-intercepts and the average value of r for the laboratory populations were much higher. In other words, the indicated cadmium toxicity was the same whether the populations on the whole were increasing, as were the laboratory populations, or decreasing, as were the natural populations.

The present study demonstrates that the results of appropriate short-term laboratory experiments using whole populations can be verified in the natural environment. Future studies to determine the relationships between short-term and long-term effects of toxicants on integrating indicators of stress (average numbers or biomass) in Daphnia populations will be useful in extrapolating the results of short-term in situ experiments to assess longer-term effects.

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