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Field and Laboratory Tests on Acute Toxicity of Cadmium to Freshwater Crayfish

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Cadmium is a non-essential, extremely toxic trace element (Hiatt and Huff 1975). Organisms such as freshwater crayfish accumulate cadmium from water and food (Giesy et al. 1980) and sequester the metal primarily in the gills and viscera (Anderson and Brower 1978). Because cadmium is not regulated by crayfish (Bryan 1976), body burdens may exceed environmental concentrations by an order of magnitude or more (Gillespie et al. 1977). Depending on water characteristics, crayfish may be relatively immune to acute exposures of 1 mg Cd/L (Gillespie et al. 1977) but die when subjected to 0.005–0.01 mg Cd/L over several months in soft water (Thorp et al. 1979).

Environmental regulatory standards for cadmium (EPA 1980), like those for most pollutants, are based on acute, laboratory toxicity tests of single species. Such tests can be conducted rapidly and inexpensively in comparison to acute or chronic field studies, but their validity has often been questioned (e.g., Kimball and Levin 1985). Laboratory-based criteria are subject to two criticisms: (1) chemical and physical conditions differ greatly in degree and variability from laboratory to field, and (2) species are not isolated, but live in an ecosystem of interacting taxa and bio-feedback. In nature a toxicant's effect on one species may be magnified, as an additive or synergistic response to other environmental conditions, or counterbalanced by the more severe decrease in populations of that species' competitors or predators.

To investigate the validity of basing field toxicity standards on laboratory data, we subjected the freshwater crayfish Orconectes immunis (Hagen 1870) for 96 h to various levels of cadmium in laboratory aquaria and experimental ponds. The study was designed to evaluate in part the first criticism of lab-based criteria (as cited above). Our studies were conducted concurrently with similar short-term experiments (Sherman 1985) on the fathead minnow, Pimephales promelas, and coincided with studies of chronic cadmium stress on fathead minnows in experimental ponds.

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MATERIALS AND METHODS

Adult and juvenile crayfish (all $\geq$ 5 cm total length) were obtained from a commercial bait supplier in central New York and acclimated in the laboratory for at least one week. During the experiment, random groups of ten crayfish (five of each sex) were placed in floating, nylon mesh bags (76 cm deep and 30 cm wide; mesh diameter ca. 5 mm). Each bag was submerged individually in an aerated 70-L aquarium. Test temperatures averaged 16.4 $\pm$ 2° C, and a 16:8 light:dark photoperiod was maintained. Dissolved oxygen (average = 9.2 mg/L) was always adequate.

We designed the experiment to determine 96-h LC_{50} values (using standard protocols as described by EPA [1975]) for crayfish subjected to two treatments: "water type" and "nominal cadmium concentration." Duplicates of each treatment combination were run, and the entire experiment was repeated one week later (thus, four replicates per treatment combination). Mortality was recorded daily. Because few crayfish died in either experiment, we exposed the animals to cadmium for an additional 96 h after completion of the second experimental run (total continuous exposure = 192 h).

Three types of water were used: (1) laboratory water, (2) pond water, and (3) pond water over sediments. Laboratory water was dechlorinated (activated carbon filtered) tap water that met EPA criteria for bioassays (EPA 1975). Pond water and sediments were obtained two days prior to the study from ponds used in our field experiments. This water was then mixed in a large fiberglass tank before being added to aquaria. We added pond sediment to a depth of three cm in half the aquaria containing pond water. In comparison to water in experimental ponds (as reported by Sherman [1985]), laboratory water was characterized by significantly lower dissolved organic carbon (1.2 vs. 9.1 mg/L) but showed no significant differences in alkalinity (86.4 vs. 100.0 mg/L as $CaCO_3$), magnesium (9.5 vs. 10.0 mg/L), and calcium (40.8 vs. 26.1 mg/L). In the winter laboratory experiments, pH values for all water types were similar (7.65 $\pm$ 0.5). The ponds had an average pH of 8.6 in the field studies conducted during the preceding fall.

Crayfish were either kept in control aquaria (total cadmium < 0.001 mg/L) or subjected to one of the following nominal concentrations of cadmium: 1.0, 1.8, 3.2, 5.6, or 10.0 mg/L. Cadmium solutions were made from reagent grade $CdCl_2$. Actual values of cadmium in solution were determined on a daily basis from samples taken from each treatment combination and analyzed with flame and flameless atomic absorption spectrometry or with a specific-ion electrode. Detailed descriptions of techniques used in analyzing concentrations and size distributions of cadmium for laboratory and field studies are given in Sherman (1985).

Field studies were conducted in 0.07-ha experimental ponds operated by the Agronomy Department at Cornell University. Each

pond had a maximum depth of 1.5 m and contained roughly 1.3 million liters of water. Twelve ponds with similar physical, chemical, and biological features were selected for the study. A nearly constant 16°C was recorded in all ponds during the 96-h experiment. Some chemical characteristics of the ponds were described in the preceding section.

As in the laboratory experiment, five male and five female Orconectes immunis were enclosed in floating, nylon mesh bags. Five bags were placed in each experimental pond. Mortality data, recorded daily from these within-pond replicates, were averaged to increase the accuracy of the individual-pond mortality datum. Enough cadmium was added to nine of the ponds (selected randomly) to yield three nominal levels of cadmium: 2.91, 3.52, or 4.39 mg/L. The remaining three ponds received no cadmium and served as reference ponds (thus, three replicated ponds for each treatment level and reference). Immediately before crayfish were added, cadmium was sprayed on the surface of each pond from a 400-L, truck-mounted tank containing concentrated $CdCl_2$ in pond water. The nominal cadmium levels corresponded to the laboratory LC_{50} values for fathead minnows in pond water, pond water with sediments, and laboratory water, respectively, as determined in an earlier, related study by Sherman (1985). [The principal taxon in the field study was the fathead minnow; hence, the design of the field project with crayfish had to conform for practical reasons to the treatment levels in the fish study. The laboratory study of crayfish was conducted shortly after the field study.] Actual levels of cadmium were measured every 4 h during the first 24 h and daily thereafter.

RESULTS AND DISCUSSION

Our laboratory study was designed to answer two basic questions. First, "Was crayfish mortality related to the concentration of cadmium in the water?" And second, "Did characteristics of the water affect mortality from cadmium?" It is apparent from Table 1 that juvenile and adult males and females of this crayfish species tolerate acute exposures to at least 10 mg/L of cadmium under the environmental conditions prevailing in this study. There were no significant differences in absolute mortality among cadmium treatments and controls (chi-square = 3.00, d.f. = 5, $P > 0.05$). In fact, as many crayfish died in control aquaria as in any of the cadmium treatments. Even when the second laboratory experiment was extended an extra four days, no additional mortality occurred for any group. Likewise, the type of water and the presence or absence of sediment did not significantly affect absolute mortality (chi-square = 0.25, d.f. = 2, $P > 0.05$), although this difference might have been evident if cadmium concentrations had been greater and total mortality higher.

Absolute crayfish mortality in the field experiment was not related to nominal levels of cadmium in the ponds (chi-square = 1.11, d.f. = 3, $P > 0.05$). The percent mortality was slightly lower in the laboratory (Table 1) than in the ponds (Table 2).

Table 1. 96-h mortality of crayfish in laboratory aquaria dosed with cadmium. Values are averages from four replicated aquaria with five male and five female crayfish/aquarium are given in percentages x 100.

Nominal Cadmium Concentration (mg/L)	Water Type: Lab Water	Pond Water	Pond Water & Sediments	Row Averages
Control (0.0)	2.5	0	0	0.83
1.0	2.5	0	2.5	1.67
1.8	0	0	0	0
3.2	2.5*	0	2.5	1.67
5.6	0	2.5	2.5*	1.67
10.0	0	2.5	0	0.83
Column Averages	1.25	0.83	1.25	-

* One dead crayfish was a female.

Average mortality in the field was greatest in reference ponds. This suggests that most, if not all, deaths could be attributed to handling stress on crayfish (which necessarily was greater in the field than in the laboratory) rather than to the expected cadmium toxicity.

The ratio of actual to nominal concentrations of cadmium decreased more rapidly in ponds than in the laboratory. [Chemical results discussed here are derived from joint field experiments and concurrent laboratory studies with chemically identical conditions, as reported in Sherman (1985).] Within four hours, 50-60% of the total cadmium in the ponds occurred as Cd^{++}, but this component was reduced to 5-15% at the end of 24 h, as the result of rapid precipitation of $CdCO_{3(s)}$. The concentration of Cd^{++} diminished to 0.1-0.2 mg/L within a day and did not reach equilibrium by the end of the 96-h experiment. Similar, but more gradual, trends were observed in the laboratory.

Cadmium is toxic to all crustaceans, although LC_{50} values are usually much lower for freshwater than marine benthic species. Depending on the species, acute LC_{50s} for marine benthic crustaceans have varied from 0.32 to 16.7 mg Cd/L (Eisler 1971, Ahsanullah 1976) but are usually above 1 mg/L. For freshwater taxa, 96-h median lethal concentrations of $CdSO_4$ were 0.06 mg/L for the shrimp Paratya tasmaniensis and 0.04 mg/L for the amphipod Austrochiltonia subtenuis (Thorp and Lake 1974). Crayfish seem to be relatively more tolerant of cadmium than are smaller crustaceans (for data on planktonic crustaceans, see Marshall 1978, Kettle et al. 1980). Gillespie et al. (1977) reported no significant mortality to Orconectes propinquus when it was subjected to acute concentrations as high as 1 mg/L, even though body burdens occasionally exceeded 500 ppm after eight days. Likewise, we found no significant mortality of Orconectes immunis in either laboratory or field experiments with hard water

(approximately 100 mg/L as $CaCO_3$). In soft water (about 10 mg/L as $CaCO_3$), only 20% of a population of the crayfish Cambarus latimanus died within five months after exposure in flow-through tanks to chronic doses of 0.01 mg Cd/L, although all crayfish in adjacent mesocosms died within one year (Thorp et al. 1979).

Table 2. 96-hour mortality of crayfish in experimental ponds dosed with cadmium. Values are averages from five replicated bags/pond with five male and five female crayfish in each bag.

Treatment Level (Nominal Cd conc. in mg/L)	Pond* No.	Mortality (% x 100)	Highest Recorded Cd Conc. (mg/L)@
Reference (0.00)	223	6.0	< .001
"	224	6.0	< .001
"	232	10.0	< .001
"	Average	7.3	< .001
2.91 mg/L	233	4.0	2.26
"	236	4.0	2.47
"	238	6.0	2.20
"	Average	4.7	2.31
3.52 mg/L	225	8.0	2.26
"	228	6.0	2.79
"	229	2.0	2.67
"	Average	5.3	2.57
4.39 mg/L	227	10.0	3.87
"	231	8.0	4.33
"	234	2.0	2.75
"	Average	6.7	3.65

* Numbers designate experimental ponds at Cornell University.
@ Data from Sherman (1985)

Crustaceans are generally more sensitive than fish to toxic heavy metals although this trend depends somewhat on the species studied (e.g., Eisler 1971, Thorp et al. 1979). In our experiments, however, this pattern was not evident. The LC_{50s} for the fathead minnow were 2.0-4.4 mg Cd/L in the laboratory (Sherman 1985), levels which crayfish easily tolerated. This concentration compares with a 96-h median tolerance limit of about 55 mg Cd/L for fathead minnows in another experiment with hard water (360 mg/L as $CaCO_3$) described by Pickering and Henderson (1969). In contrast, mortalities did not exceed 10% in combined field experiments with fathead minnows (Sherman 1985) and crayfish, and the number of deaths for both taxa were often greater in the reference ponds than in the cadmium-treated ponds.

What can account for the low mortality in our field and laboratory experiments? The answer to this question involves the chemistry of cadmium in our systems. Toxicity of cadmium is related to the availability of the free cadmium ion rather than to the total amount of cadmium in solution (e.g., Sunda et al 1978). The

concentration of Cd^{++} is affected by water hardness, alkalinity, organic and inorganic ligands (e.g., Giesy et al. 1977), and possibly pH (Sherman 1985). In our experiments, toxic Cd^{++} was reduced to < 1 mg/L within 24 h by its conversion to nontoxic $CdCO_3$ precipitate in the slightly alkaline, hard water of both the laboratory and field (Sherman 1985).

If relationships between environmental chemistry and metal toxicity are known, is the use of laboratory-based criteria justified for setting permissible levels of environmental pollutants? Similar questions asked for over a decade (e.g., Geckler et al. 1976, Marshall 1978, Kettle et al. 1980, Hansen and Garton 1982, Kimball and Levin 1985) have yielded no consistent answers. Our results with crayfish support the use of laboratory-based criteria for predicting acute toxicities in the field, with the important caveat that this conclusion could have been different if treatments and controls had varied significantly and if mortalities had been greater. In other studies where environmental chemistry was considered, laboratory bioassays accurately predicted toxicities to stream and lake biota from copper (Geckler et al. 1976), cadmium (Marshall 1978), and the insecticide diflubenzuron (Hansen and Garton 1982). However, laboratory results poorly predicted field toxicities to cladocerans (Kettle et al. 1980) and fathead minnows (Sherman 1985). In addition, single-species laboratory tests with fish and invertebrates have been less effective in predicting community-wide effects (e.g., Hansen and Garton 1982).

In general, therefore, acute tests in the laboratory are somewhat useful (albeit not infallible) in predicting effects of acute exposures to single species in the field. Still lacking at this stage is an adequate understanding of how the results of acute, single-species laboratory experiments relate to the chronic effects on individual taxa in the field or to the acute and chronic effects on communities and ecosystems.

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