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THE INDUCTION OF TOLERANCE TO HEAVY METALS IN NATURAL AND LABORATORY POPULATIONS OF FISH

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ABSTRACT

Aquatic toxicity studies were performed on two natural populations of fathead minnows. One group of organisms was taken from a metal-contaminated flyash pond associated with a coal-fired power plant and the other group was collected from relatively uncontaminated hatchery ponds. Acute tests indicated that flyash pond fish were significantly more tolerant to cadmium and copper than were hatchery fish. At an exposure concentration of 6.0 mg Cd/L in moderately hard water, the median period of survival for flyash pond fish was 50.0 hr compared to 6.8 hr for hatchery fish. Both groups of organisms were about equally sensitive to zinc. The metal-induced tolerance observed with animals from the flyash pond was not a sustained response. Additional studies were undertaken to observe the responses of laboratory populations of fathead minnows to acutely toxic cadmium concentrations following acclimation to sublethal exposures of this metal. Based on 96-hr LC_{50} values, those animals which had received 35-days prior exposure to 10 and 50 μg Cd/L were 63 to 68% more tolerant to cadmium than were previously unexposed organisms. As with the natural population, tolerance to cadmium in the laboratory fish was not retained. After organisms which had been acclimated to 10 μg Cd/L were transferred to clean water, tolerance to cadmium decreased by three and one-half fold after only 7 days. Developing embryos of the fathead minnow and rainbow trout also acquired tolerance to cadmium. After eggs of the trout had been exposed to 0, 5, and 50 μg Cd/L for 24 days, subsequent acute toxicity tests conducted on the newly hatched larvae gave 7-day LC_{50} values of 0.70, 1.59, and 2.02 μg Cd/L, respectively.

Descriptors: Cadmium
Copper
Embryos
Larvae
Metals
Water Quality
Zinc

Indicators: Acclimation
Aquatic Toxicity Tests
Bioaccumulation
Depuration
Fathead Minnow
Natural Populations
Rainbow Trout
Tolerance

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CHAPTER I

INTRODUCTION

The release of heavy metals into aquatic environments as a consequence of industrialization and urbanization has created numerous environmental and human-health problems. The continued occurrence of acid deposition (Haines, 1981) and widespread atmospheric contamination by heavy metals (Hirao and Patterson, 1974; Van Loon and Beamish, 1977) indicate that future problems will involve diffuse sources. Increased dependence on coal and other fossil fuels as national energy sources also creates prospects for further environmental contamination, particularly for water resources. Freshwater systems constitute the major repository for most coal-derived elements, and upwards of sixty inorganic aquatic pollutants have been traced to coal (Vitez, 1976). Unlike petroleum hydrocarbons, which are subject to biodegradation, metals are persistent in the environment. The magnitude of these effects is influenced by the elasticity of the exposed community (Cairns, 1976).

The extent to which natural fish populations can acclimate to chronic low-level exposure to heavy metals has not been fully investigated. This is surprising as resulting changes in tolerance may be of considerable importance in hazard assessment, particularly as applied to energy technologies and the development of site-specific criteria for aquatic life. Traditionally, laboratory experiments designed to test the effects of sublethal toxicant concentrations involve fish that have been held in clean water. However, the results of such experiments may not be applicable to the establishment of water quality criteria for natural fish populations already exposed to low-level pollution stress.

Documentation concerning heavy metal tolerance among natural populations has been restricted to aquatic plants and invertebrates (Mierle and Stokes, 1976; Fisher and Frood, 1980; Fraser, 1980). Although pesticide resistance has been shown in fish populations (Chambers and Yarbrough, 1979), no examples of metal tolerance have been reported. Because metal contaminants continue to be a problem in aquatic systems and may become even more widespread in conjunction with acid deposition, information on whether fish can adapt to these toxicants is needed.

The present investigation was undertaken first to determine if and to what extent natural fish populations adapt to chronic metal pollution stress, and second to determine the extent to which animals maintain acclimation-induced changes in metal tolerance. To achieve this, tolerance to metals was compared between populations of fathead minnows taken from a metal-contaminated coal-fired power plant flyash pond and a less contaminated fish hatchery pond. In addition to these studies, the induction of tolerance in laboratory populations of fish (*i.e.*, fathead minnows, rainbow trout) under controlled conditions was examined. Both adult and early-life stages were used in this segment of the investigation.

CHAPTER II

RESEARCH PROCEDURES

Selection of animal species. Acute toxicity tests were conducted using environmentally exposed and unexposed adult fathead minnows (*Pimephales promelas*) to quantify their sensitivity to various heavy metals. The unexposed animals were procured from hatchery ponds located at the EPA Newtown Fish Toxicology Laboratory, Newtown, Ohio. Environmentally exposed fathead minnows were obtained from a local 40-acre power plant flyash pond. The fathead minnow population in the flyash pond has been under observation for the past five years. Despite prolonged exposure to toxic metals, this population has remained healthy and has retained significant reproductive potential.

Embryo-larval toxicity tests with cadmium were performed using the rainbow trout (*Salmo gairdneri*) and fathead minnow. These species were chosen for their economic importance, seasonal availability, egg production, and for variations in ecological and geographic distribution, including warm-water and cold-water habitats. Gravid rainbow trout were procured from the Wytheville National Fish Hatchery, Wytheville, Virginia. Eggs and sperm were obtained by the artificial milking and spawning procedures of Leitritz and Lewis (1976). Fertilization was accomplished by mixing eggs and milt for 20 minutes. Freshly fertilized fathead minnow eggs were collected from established laboratory cultures.

Selection of toxicants. Using data from several recent investigations (Chu, *et al*., 1978; Pellizzari, 1978; Torrey, 1978), the composition of coal and ash pond effluent were compared for 15 of the elements which could prove detrimental to aquatic life (Table 1). Because of their relatively high levels of occurrence in coal and flyash effluents and their importance as environmental contaminants, cadmium, copper, and zinc were selected for study. All three metals have been designated by EPA as priority pollutants (Keith and Telliard, 1979). Certified ACS grade cadmium chloride ($CdCl_2 \cdot 2\frac{1}{2}H_2O$), copper sulfate ($CuSO_4 \cdot 5H_2O$), and zinc chloride ($ZnCl_2$) used for testing were obtained from the Fisher Scientific Company.

Table 1. Concentrations of toxic elements in coal and ash pond effluent.[1]

Element	Coal (mg/kg)	Ash Pond Effluent (mg/L)
Aluminum	4300 - 30400	1.4 - 7.2
Arsenic	0.5 - 106	0.005 -0.038
Barium	33 - 150	0.1 - 0.3
Beryllium	0.2 - 31	< 0.01 - 0.01
Cadmium	0.1 - 65	0.001 - 0.037
Chromium	0.3 - 610	0.004 - 0.067
Copper	1.8 - 185	0.01 - 0.31
Lead	4 - 218	0.01 - 0.06
Magnesium	100 - 2500	0.4 - 14
Manganese	6 - 181	0.01 - 0.58
Mercury	0.01 - 1.6	0.0002 - 0.038
Nickel	0.4 - 104	0.05 - 1.1
Selenium	0.4 - 7.7	0.002 - 0.065
Silver	0.03 - 0.19	< 0.01 - 0.01
Zinc	15 - 5600	0.03 - 1.51

[1]Data taken from Chu, et al. (1978), Pellizzari (1978), Torrey (1978).

Acute toxicity tests using natural fish populations. Fathead minnows, approximately 40 mm in length, were seined from the Newtown hatchery and flyash ponds, brought into the laboratory, and held in their respective natural waters for 10 days prior to toxicity testing. Selected chemical characteristics of the hatchery and ash pond waters are given in Table 2. Fiberglass tanks, with an approximate capacity of 200 L, were used for acclimation. Following acclimation, static 96-hr tests with cadmium, copper, and zinc were performed using glass, 20-L exposure chambers. The chambers were provided with moderate aeration, and tests were conducted in a temperature range of 21 to 23°C. Each inorganic element was administered at five to eight exposure concentrations with a control. Toxicant stock solutions were prepared with deionized water, and test concentrations were formulated by transferring appropriate aliquots of the stock to reconstituted water. Toxicity tests were conducted in duplicate in both hard and moderately hard water, prepared using the basic formula for reconstituted water described by Birge, et al. (1981). The moderately hard water had a pH of about 7.4, a water hardness of approximately 103 mg/L as $CaCO_3$, a dissolved oxygen concentration of 7.0 mg/L, and a conductivity of 283 to 300 μmhos/cm. The hard water had similar pH and dissolved oxygen levels as above; however, water hardness ranged from 254 to 271 mg/L as $CaCO_3$, and conductivity varied between 500 and 553 μmhos/cm. The different hardness levels used in these tests corresponded to the extremes in hardness observed throughout the period of investigation on the two natural waters. Methods used for quantifying general water quality characteristics and toxicant concentrations are given below under Chemical monitoring procedures.

Additional toxicity tests were conducted to determine the extent to which animals maintained acquired tolerance. Fish from both the Newtown hatchery and flyash ponds were transferred into uncontaminated reconstituted water for 7 to 14 days before the initiation of testing. In this series of experiments, care was taken to test fish in water of the same hardness to which the organisms had been exposed in the environment. This was necessary in order to avoid confusing loss of tolerance by the organism with any biological and/or chemical effects of water hardness on metal toxicity (Calamari, et al., 1980). Moderately hard water was used in these studies with Newtown animals and hard water was used with fish from

Table 2. Selected chemical characteristics of water from Newtown hatchery and flyash pond.[1]

Characteristic	Newtown Hatchery		Flyash Pond	
	Mean	Range	Mean	Range
Cadmium (μg/L)	0.28	0.09 - 0.52	0.46	0.30 - 0.98
Copper (μg/L)	1.50	0.87 - 2.50	4.38	2.29 - 6.89
Zinc (μg/L)	3.80	2.00 - 7.00	7.00	6.00 - 8.00
pH	7.8	7.6 - 8.2	7.5	7.2 - 7.8
Hardness (mg/L as $CaCO_3$)	229	140 - 320	224	176 - 256
Conductivity (μmhos/cm)	430	356 - 669	409	356 - 488

[1]Values for metals were obtained on water samples passed through a 0.45-μm membrane filter.

the flyash pond.

Acclimation to cadmium by adult fathead minnows. Studies were conducted to investigate the differing degrees of cadmium tolerance exhibited by the Newtown fathead minnows following acclimation to sublethal cadmium concentrations for varying periods of time. Fish were held in charcoal-filtered tap water for a minimum of 4 weeks prior to cadmium exposure. This water had a mean value of 130 mg $CaCO_3$/L for hardness, 254 μmhos/cm for conductivity, 8.2 mg/L for dissolved oxygen, 70 mg $CaCO_3$/L for alkalinity, and 7.6 for pH. Following the holding period, separate populations of fathead minnows were acclimated to 0, 1, 10, and 50 μg Cd/L for 35 days. An additional group of animals was acclimated to progressively increasing cadmium concentrations; fish were exposed to 10 μg Cd/L for 7 days, 50 μg Cd/L for the following 14 days, and 100 μg Cd/L for an additional 14-day period (i.e., 10→50→100 μg Cd/L). Exposure was carried out using 200-L fiberglass acclimation tanks. A continuous flow of charcoal-filtered tap water was supplied to each tank. Cadmium solutions were prepared in deionized water, acidified to a pH of 2, and delivered to acclimation tanks via Sage syringe pumps (Model 355) fitted with double-ground glass syringes. Exposure levels were regulated by varying the concentration of toxicant delivered from the syringe pump. Dilution water flow rates were monitored using Gilmont flow meters and timed volumetric measurements. The flow rate was set at 9 L/hr, giving one volume addition every 24 hr. The loading capacity of the acclimation tanks was under the limit of 1 g fish/L water as recommended by ASTM (1981). Fish were fed during acclimation and maintained on a 16-hr light/8-hr dark photoperiod. Water temperature ranged between 20 and 22°C. After 35 days, fish were removed from the acclimation tanks and used in static 96-hr toxicity tests with cadmium. Tests were conducted as described above, except that charcoal-filtered tap water instead of reconstituted water was used for dilution.

Fish not included in the above toxicity tests were removed from the cadmium acclimation conditions after 35 days and exposed to clean water. Acute tests with cadmium were then initiated on these organisms 7 to 21 days after termination of metal acclimation to determine any loss of tolerance with time.

Acclimation to cadmium by fish embryo-larval stages. The final phase of this project was undertaken to determine if tolerance could be induced in early life-cycle stages of fish. To accomplish this objective, freshly fertilized eggs of the fathead minnow and rainbow trout were exposed to 0, 5, and 50 μg Cd/L for 4 and 24 days, respectively. These periods represented the embryonic developmental times for each species. Within 24 hrs of egg hatching, 7-day toxicity tests were performed on the larval stages. Cadmium solutions prepared with reconstituted water were placed in 400-mL Pyrex deep petri dishes which served as exposure chambers. Toxicant solutions were renewed at regular 24-hr intervals. During these studies, water hardness ranged from 89 to 107 mg/L, pH, from 7.5 to 7.7, dissolved oxygen from 8.7 to 9.8 mg/L, and conductivity, from 254 to 298 μmhos/cm. Temperature was set at 14°C for the rainbow trout and 25°C for the fathead minnow.

Data analysis. Mortality was the principal test response observed in all toxicity tests. Median lethal concentrations (LC_{50}'s) were determined by log probit analysis (Finney, 1971), and significant differences between the LC_{50} values were assessed by standard error of the difference (Sprague and Fogels, 1977). Median periods of survival (LT_{50}'s) were calculated following the procedures of Litchfield (1949).

Chemical monitoring procedures. In all toxicity tests, water samples were collected daily from each exposure chamber and analyzed for total metal using a Perkin-Elmer atomic absorption spectrophotometer (AAS, model 503), equipped with a graphite furnace (model HGA 2100). In addition, total and soluble metal was measured in flyash and Newtown pond waters, as well as in the acclimation tanks. Total metal samples were acidified with nitric acid to 1% by volume and stored, if necessary, in linear polyethylene bottles. Samples for soluble metal were filtered through a 0.45-μm membrane filter prior to acidification and analysis.

Test water was monitored at regular intervals for pH, dissolved oxygen, conductivity, and water hardness using a digital pH meter (Corning model 110), oxygen meter (YSI model 51A), conductivity meter (Radiometer model DCM 2e), and the EDTA titrimetric procedure (ASTM, 1981), respectively. In a limited number of tests, alkalinity also was measured using the methyl orange titrimetric procedure (ASTM, 1981).

To determine metal concentrations in animal tissues, samples were prepared for analysis by wet digestion. Prior to AAS analysis, 100-mg tissue samples (wet weight) were pre-digested at ambient temperature with

1 ml of a 1:1 mixture of concentrated nitric and 70% perchloric acid. After 24 hours, samples were heated in a water bath at 70°C for 3 hours. Standard solutions and reagent blanks were treated in the same manner.

CHAPTER III

RESULTS AND DISCUSSION

Acute toxicity tests using natural fish populations. Selected chemical characteristics of water from the Newtown hatchery and flyash ponds are given in Table 2. General water quality characteristics (i.e., pH, hardness, conductivity) for the two water sources, while subject to seasonal fluctuations, were similar. However, metal concentrations varied significantly. Respective mean values for cadmium, copper, and zinc in the Newtown hatchery pond were 0.28, 1.50, and 3.80 μg/L, compared to concentrations of 0.46, 4.38, and 7.00 μg/L in the flyash water. Correspondingly, whole-body metal concentrations were two to four times greater in the flyash pond animals than in the Newtown hatchery animals (Table 3). Although the Newtown hatchery animals were exposed to relatively low levels of copper and zinc, they were essentially naive to cadmium exposure. The concentrations of cadmium in the whole body (0.04 μg/g) and internal organs (0.04 μg/g) of the hatchery animals were close to the cadmium detection limit (0.02 μg/g). By comparison, the whole body and internal organs of the flyash animals contained 0.15 and 0.51 μg Cd/g, respectively. Respective mean values for cadmium in the gill tissue of Newtown and flyash fish were 0.12 and 0.35 μg/g.

Fathead minnows from both sources were exposed to the three test metals at each of two water hardness levels, and the 24-hr and 96-hr LC_{50} values are reported in Table 4. The 24-hr LC_{50}'s determined with the two fish populations were significantly different from each other for both cadmium and copper administered in moderately hard water. For example, cadmium exposure resulted in 24-hr LC_{50} values of 4.03 and 6.06 mg/L with the Newtown and flyash animals, respectively. However, after 96 hr exposure, no significant differences in response were observed between the two populations. With copper, the greater sensitivity of the Newtown fish was also more readily apparent at 24 hr. Although no significant differences in sensitivity were observed between the two populations to either cadmium and copper in hard water, the flyash pond fish appeared to demonstrate greater tolerance than Newtown animals. However, results of

Table 3. Tissue metal concentrations in fathead minnows from the Newtown hatchery and flyash pond.

Metal	Tissue	Metal Concentration (μg/g)[1]	
		Newtown Hatchery	Flyash Pond
Cadmium	Whole body	0.04 ± 0.01	0.15 ± 0.03
	Internal organs	0.04 ± 0.01	0.51 ± 0.15
	Gills	0.12 ± 0.03	0.35 ± 0.08
Copper	Whole body	0.28 ± 0.03	0.54 ± 0.09
	Internal organs	1.00 ± 0.17	1.95 ± 0.27
	Gills	0.59 ± 0.09	1.28 ± 0.14
Zinc	Whole body	34.2 ± 4.9	70.5 ± 4.7
	Internal organs	30.6 ± 0.8	65.2 ± 4.0
	Gills	50.2 ± 3.9	89.5 ± 7.6

[1]Expressed as mean ± standard error.

Table 4. Acute toxicity of metals to Newtown hatchery and flyash pond fathead minnows.

Metal	Nominal Water Hardness (mg/L as $CaCO_3$)	Exposure Time (hr)	Newtown Hatchery		Flyash Pond	
			LC_{50} (mg/L)	95% Confidence Limits	LC_{50} (mg/L)	95% Confidence Limits
Cadmium	100	24	4.03	2.71 - 4.99	6.06	5.12 - 7.29
		96	3.06	2.00 - 3.81	3.89	3.23 - 4.47
	250	24	9.27	7.51 - 11.31	12.58	4.96 - 17.08
		96	7.16	5.92 - 8.81	9.55	5.72 - 12.10
Copper	100	24	0.27	0.20 - 0.36	0.46	0.25 - 0.56
		96	0.21	0.16 - 0.28	0.36	0.23 - 0.45
	250	24	0.72	0.54 - 1.15	0.76	0.42 - 1.34
		96	0.39	0.26 - 0.54	0.41	0.12 - 0.56
Zinc	100	24	6.88	6.21 - 7.92	6.90	6.23 - 7.55
		96	6.09	5.57 - 6.45	6.14	5.48 - 6.98
	250	24	7.62	6.82 - 10.19	8.49	5.51 - 11.61
		96	7.45	6.67 - 9.89	5.96	4.16 - 7.84

the acute tests with zinc indicated that both populations were about equally sensitive to this metal.

While the LC_{50} data were sufficient to demonstrate trends in tolerance, the differences in response were more dramatic when median period of survival values (LT_{50}) were calculated (Table 5). Significant differences were observed between the Newtown and flyash fish populations with both cadmium and copper. At an exposure concentration of 6.0 mg Cd/L in moderately hard water, the LT_{50} for Newtown hatchery animals was 6.8 hr compared to 50.0 hr for flyash pond animals. Exposures to 16.0 mg Cd/L in hard water resulted in cadmium LT_{50} values of 3.7 hr for Newtown fish and 13.5 hr for flyash pond animals. Survival periods of flyash pond animals following exposures to copper in both types of test water were two to four times longer than those observed for Newtown animals. No consistent trends in tolerance were noted for fish populations exposed to zinc.

The tolerance of flyash pond minnows to cadmium and copper was not a sustained response. After fish from the flyash pond had been transferred to reconstituted water, there was a progressive decrease in 96-hr LC_{50} values relative to those observed with Newtown animals (Table 6). Linear regression analysis of the LC_{50} values at 0, 7, and 14 days for flyash pond fish gave negative slopes which indicated a continuing decrease in tolerance with time to both cadmium ($m = -0.217$) and copper ($m = -0.015$). From the values presented in Table 6, the loss of tolerance with the flyash pond minnows was evident after only 7 days in clean water. On the other hand, the nearly zero slopes derived from the LC_{50}'s with Newtown animals indicated relatively no change in susceptibility to either cadmium or copper with time.

Acclimation-induced changes in response of laboratory populations of fathead minnows to cadmium. Studies were undertaken to investigate the differing degrees of cadmium tolerance exhibited by laboratory populations of fathead minnows following 35 days of acclimation to several sublethal cadmium concentrations. Survival frequencies during this period were 99% to 100% for acclimation exposure levels of 0, 1, and 10 μg Cd/L, 96% at 50 μg Cd/L, and 98% at 10→50→100 μg Cd/L. Whole-body cadmium concentrations in fathead minnows acclimated to the various levels of aqueous metal increased markedly with time during this period (Figure 1). Relative to controls, the body burden of cadmium increased by 8-, 230-, 187-, and 242-

fold after 35 days exposure to 1, 10, 50, and 10→50→100 μg Cd/L, respectively.

Pretreatment with 10 μg Cd/L resulted in significant increases in acclimation-induced tolerance to cadmium by laboratory populations of fathead minnows. At this acclimation level after 35 days, the subsequent 96-hr LC_{50} was significantly greater than that determined with the control animals (Table 7). Furthermore, compared to controls, LC_{50} values were 20% and 63% higher following 35-day exposures to 10→50→100 and 50 μg Cd/L, respectively (Table 7). Although these increases were not sufficiently large to show any statistical significance, the trends for increasing tolerance were apparent. By comparison, animals acclimated to 1 μg Cd/L were somewhat more sensitive than the control group.

Acclimation-induced tolerance to cadmium did not appear to be a sustained response. For example, when fish were returned to control water for 7 days, after the 35-day exposure period to cadmium, the LC_{50} at the 10 μg Cd/L acclimation concentration decreased from 2.88 to 0.83 mg/L (Table 7). This greater toxicity correlated with a substantial reduction in the cadmium body burdens during the same period (Figure 1). Additional toxicity tests were conducted after 14 and 21 days of depuration, but most of the tolerance loss was observed during the first 7 days.

Acclimation-induced changes in response of early-life stages of fish to cadmium. Investigations were performed to study metal tolerance exhibited by embryo-larval stages of fathead minnows and rainbow trout. As noted above, embryonic stages of the minnow and trout were acclimated to 0, 5, and 50 μg Cd/L for 4 and 24 days, respectively. Survival frequencies during the acclimation period ranged from 57 to 90%, except at the 50 μg/L concentration at which no fathead minnows survived. Following acclimation, 7-day toxicity tests were conducted on newly-hatched fry, and LC_{50}'s are given in Table 8. Based on these values, trout and minnows which had been exposed to 5 μg Cd/L and trout which had been exposed to 50 μg Cd/L showed statistically increased tolerance over controls.

Significance of fish tolerance in aquatic hazard assessment. The natural population of fathead minnows inhabiting the flyash pond provided an example of not only the assimilative capacity of aquatic organisms but also the elasticity of natural aquatic communities. Due to the high concentration of leachable inorganic elements in flyash (Birge, 1978), organisms inhabiting the pond receive chronic exposure to toxic heavy metals. Despite this prolonged exposure, the minnow population in the flyash pond has remained healthy and has retained significant reproductive potential. This population showed the ability to tolerate subsequent exposures to normally lethal concentrations of cadmium and copper. However, no increased tolerance was observed for zinc. The lack of cross-tolerance (i.e., tolerance to one toxicant resulting in increased tolerance to another) appears to be consistent with findings in the literature. For example, copper-resistant daphnids (LeBlanc, 1982) and rainbow trout (Dixon and Sprague, 1981) were not found to be tolerant of zinc. These findings indicate that the mechanisms responsible for metal tolerance in aquatic organisms may be specific.

There is evidence that genetic factors rather than sublethal toxicant exposures may be responsible for organismal tolerance to environmental contaminants. For example, laboratory populations of fathead minnows were shown to be less tolerant to hydrogen sulfide than natural populations, although neither group had any previous exposure to this compound (Smith, et al., 1976). The relaxation of normal selection pressures in cultured populations and resultant declines in vigor may be attributed to the greater susceptibility of the laboratory populations to toxicant stress. However, in the present investigation, acclimation-induced tolerance by fathead minnows to cadmium was demonstrated under laboratory control conditions. In addition, both natural and laboratory populations of fish lost their acquired tolerance to metals after transfer to metal-free water. This may indicate that there is a biological mechanism in the fish which functions to mitigate the toxic effects of metals and that a minimum level of metal is required to activate that mechanism. Several earlier investigations have demonstrated that exposure of fish to low concentrations of metal stimulates the synthesis of metallothionein, a protein which binds with metals to produce an inactive complex (Nöel-Lambot, et al., 1978; Bouquegneau, 1979; Takeda and Shimizu, 1982; Woodworth, et al., 1983).

Studies designed to examine the differing degrees of cadmium tolerance exhibited by laboratory populations of adult fish indicated that the magnitude of effect was dependent on the concentration used for acclimation. The threshold for acclimation-induced tolerance was observed to fall at 10 μg Cd/L. Fish acclimated to concentrations at or above this threshold developed increased tolerance. However, animals acclimated to 10→50→100 μg Cd/L were not as tolerant as those acclimated to 10 and 50 μg Cd/L. The increased tolerance of animals acclimated to sublethal cadmium concentrations suggests the induction of an adaptive mechanism sufficient to mitigate the toxic effects of exposure to subsequently higher levels of metal.

Pascoe and Beattie (1979) demonstrated that acclimation of rainbow trout alevins with cadmium provided the fish with some degree of protection when they were later exposed to higher cadmium concentrations. Results of the present embryo-larval studies are consistent with those from the above investigation. However, a further and perhaps more significant finding was that the embryo-larval stages of fish were more sensitive to the effects of cadmium acclimation than were the adults. For example, acclimation to 50 μg Cd/L resulted in increased tolerance of the adult animals, whereas fathead minnow embryo-larval stages were unable to survive this level of exposure.

Table 5. Median period of survival values for metals administered to Newtown hatchery and flyash pond fathead minnows.

Metal	Nominal Water Hardness (mg/L as $CaCO_3$)	Calculated Metal Exposure Concentration (mg/L)	Newtown Hatchery		Flyash Pond	
			LT_{50} (hr)	95% Confidence Limits	LT_{50} (hr)	95% Confidence Limits
Cadmium	100	6.00	6.8	4.3 - 10.8	50.0	29.4 - 85.0
	250	16.0	3.7	3.4 - 6.4	13.5	11.7 - 15.5
Copper	100	0.50	4.5	3.0 - 6.7	17.0	11.3 - 25.7
	250	1.50	3.6	2.7 - 4.8	8.6	6.7 - 11.1
Zinc	100	7.00	14.0	9.3 - 21.0	10.5	6.4 - 17.3
	250	15.0	2.2	1.8 - 2.7	5.5	3.8 - 7.9

Table 6. Loss of tolerance to metals following transfer of fish from their natural water source into reconstituted water.

Metal	Days in Reconstituted Water Before Testing	Newtown Hatchery		Flyash Pond	
		96-hr LC_{50} (mg/L)	95% Confidence Limits	96-hr LC_{50} (mg/L)	95% Confidence Limits
Cadmium	0	3.06	2.00 - 3.81	9.55	5.72 - 12.10
	7	2.90	1.53 - 3.70	7.35	1.40 - 8.59
	14	3.10	-	6.51	3.90 - 7.70
Copper	0	0.21	0.16 - 0.28	0.41	0.21 - 0.56
	7	0.31	0.21 - 0.41	0.25	-
	14	0.12	0.09 - 0.16	0.20	0.09 - 0.28

Table 7. Toxicity of cadmium to adult stages of fish previously exposed to sublethal cadmium concentrations.

Calculated Acclimation Exposure Concentration (μg/L)	Actual Acclimation Exposure Concentration (μg/L)	Percent Survival after Acclimation	96-hr LC_{50} for Cd after the 35-day Acclimation Period (mg/L)	95% Confidence Limits	96-hr LC_{50} for Cd after the 7-day Depuration Period (mg/L)	95% Confidence Limits
0	ND[1]	100	1.71	1.15 - 2.19	1.39	0.77 - 2.43
1	0.7 ± 0.1	99	1.41	1.08 - 1.86	1.50	0.97 - 2.26
10	12.4 ± 0.3	99	2.88	2.07 - 3.72	0.83	0.36 - 1.44
50	32.4 ± 1.0	96	2.79	1.61 - 5.16	1.98	1.21 - 2.99
10 → 50 → 100[2]	92.8 ± 1.6[3]	98	2.05	1.04 - 2.82	1.51	0.48 - 2.69

[1]Not detected.

[2]Fish initially were acclimated to 10 μg Cd/L for 7 days, 50 μg Cd/L for the following 14 days, and 100 μg Cd/L for an additional 14-day period.

[3]Exposure concentration shown was measured over a 14-day period at the nominal level of 100 μg Cd/L.

Table 8. Acute toxicity of cadmium to newly-hatched fish which had been acclimated to cadmium during embryonic development.

Species	Calculated Acclimation Exposure Concentration[1] (μg/L)	Actual Acclimation Exposure Concentration (μg/L)	Percent Survival after Acclimation	7-day LC_{50}[2] (mg/L)	95% Confidence Limits
Fathead Minnow (*P. promelas*)	0	ND[3]	90	0.20	0.18 - 0.22
	5	5.6 ± 0.4	75	0.54	0.50 - 0.57
	50	50.4 ± 0.2	0	-	-
Rainbow Trout (*S. gairdneri*)	0	ND[3]	70	0.70	0.31 - 1.22
	5	5.9 ± 0.2	58	1.59	1.07 - 2.10
	50	43.2 ± 0.2	57	2.02	1.50 - 2.56

[1] Concentration to which fathead minnow and rainbow trout embryos were exposed for 4 and 24 days, respectively.

[2] Acute cadmium LC_{50} value determined with newly-hatched larvae subsequent to acclimation.

[3] Not detected.

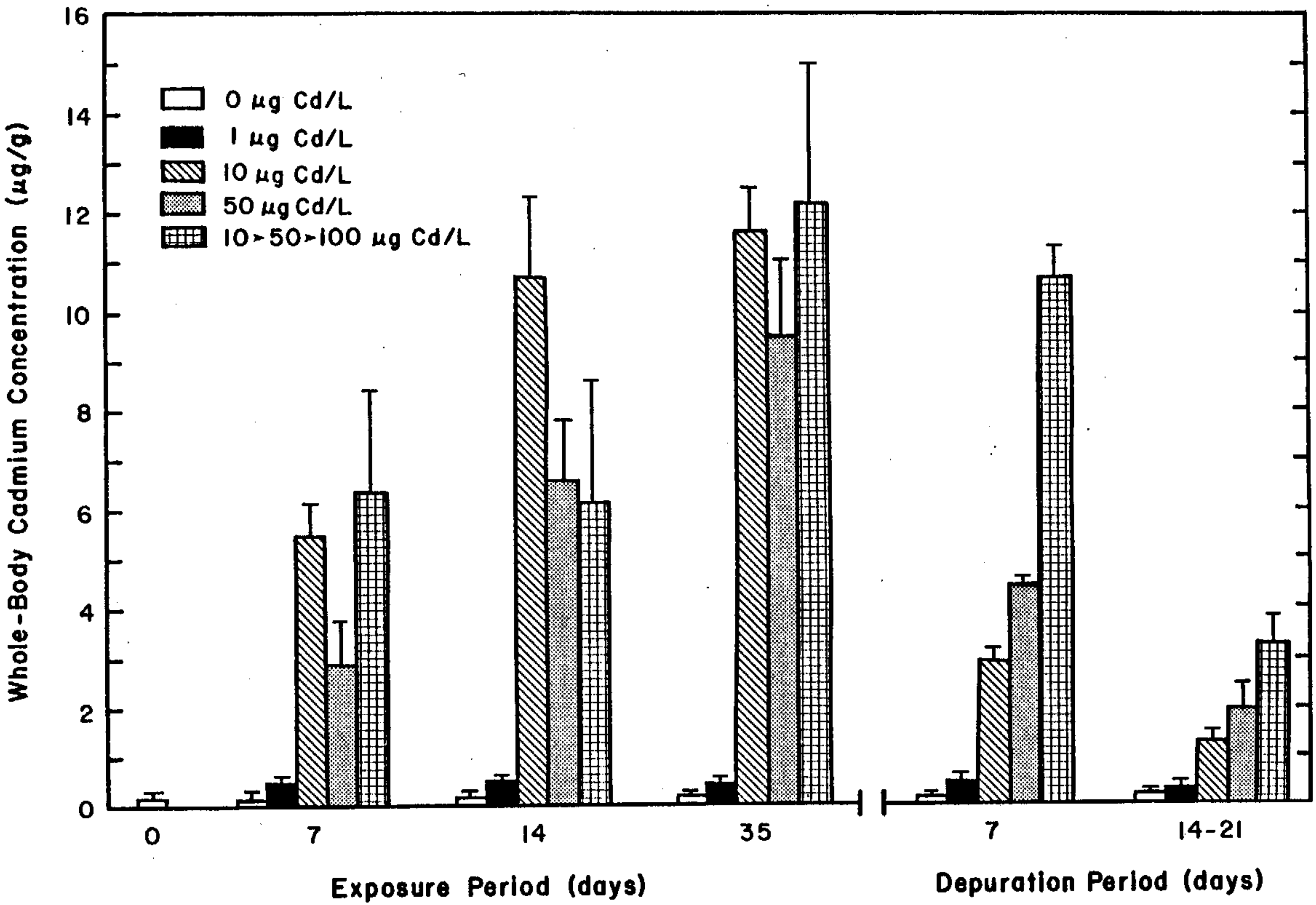

Figure 1. Whole-body cadmium concentrations in fathead minnows acclimated to various concentrations of metal. Mean values with standard error bars are given for the 35-day exposure and 21-day depuration periods. Cadmium concentrations given at 14-21 days of depuration are all 21-day values except for the 10 → 50 → 100 µg Cd/L acclimation exposure level.

CHAPTER IV

CONCLUSIONS

Aquatic toxicity studies were performed on two natural populations of fathead minnows. One group of organisms was taken from a metal-contaminated flyash pond associated with a coal-fired power plant and the other group was collected from less contaminated hatchery ponds. Acute tests with these animals indicated that flyash pond fish were significantly more tolerant to cadmium and copper than were hatchery fish. At an exposure concentration of 6.0 mg Cd/L in moderately hard water, the median period of survival for flyash pond fish was 50.0 hr compared to 6.8 hr for hatchery fish. Both groups of organisms were about equally sensitive to zinc. The metal-induced tolerance observed with animals from the flyash pond was not a sustained response. After these fish had been placed in uncontaminated water for 14 days, tolerance to cadmium and copper decreased markedly.

Additional studies were undertaken to observe the responses of laboratory populations of fathead minnows to acutely toxic cadmium concentrations following acclimation to sublethal exposures of this metal. Based on 96-hr LC_{50} values, those animals which had received 30-days prior exposure to 10 and 50 µg Cd/L were 63 to 68% more tolerant to cadmium than were previously unexposed organisms. For fish acclimated to progressively increasing metal concentrations ($10 \rightarrow 50 \rightarrow 100$ µg Cd/L), tolerance increased by 20%. As with the natural population, tolerance to cadmium decreased by a substantial degree after only 7 days. Observed decreases at this and other acclimation concentrations corresponded to appreciable reductions in metal tissue levels during the depuration period.

Developing embryos of the fathead minnow and rainbow trout also acquired tolerance to cadmium. After eggs of the trout had been exposed to 0, 5, and 50 µg Cd/L for 24 days, subsequent acute toxicity tests conducted on the newly hatched larvae gave 7-day LC_{50} values of 0.70, 1.59, and 2.02 µg Cd/L, respectively. Tolerance also increased for fathead minnows which had been exposed to 5 µg Cd/L during embryonic development.

Adaptation to chronic pollution stress can be viewed as beneficial for various animal populations. For example, in the case of an episodic discharge, biological adaptation to a pollutant or mixture of pollutants might provide protection for the existing biota. However, tolerant organisms may create possible deleterious effects to the total environment through their potential for increased bioconcentration of toxicants. In addition, natural variations in the assimilative capacity of organisms may favor the more tolerant species and, therefore, result in an ecological imbalance. Underestimating the impact of environmental contaminants undoubtedly will result in unacceptable and costly ecological degradation. On the other hand, overestimating potential hazards will place an undue economic burden on the implementation of new technologies. A better understanding of the concept of acclimation-induced tolerance is essential if we are to achieve accurate assessments on the effects of environmental pollutants.

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