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Cadmium partitioning and related effects in parasitized and non-parasitized mosquitofish (*Gambusia affinis:* Poeciliidae)

JOHN P. GIESY and DONALD H. SMITH

With 1 figure and 4 tables in the text

Introduction

The interactions of environmental stresses, both natural and man induced, have stimulated additional interest in the synergistic effects of multiple stressors on organisms. Undoubtedly, the most prolific research of this nature has been of thermal effects upon chemical toxicities in aquatic systems (CAIRNS et al. 1975). It has been shown that the presence of cestode parasites may increase the susceptibility of fish to Zn (BOYCE & YAMADA 1977) and Cd (PASCOE & CRAM 1977) toxicity. Several studies have indicated that larvae and embryos of fish are more sensitive to Cd than adult fish (BIRGE et al. 1977; EATON et al. 1978). In a study of the effects of Cd on zebra fish, SPEHAR (1976) observed inhibition of embryo production when adult female zebra fish were exposed to 8 μg Cd $\cdot$ l^{-1}. Thus, while low concentrations of metals, such as Cd, may not be acutely or chronically toxic to adult fish, populations can be adversely affected by reduced reproduction (BRUNGS 1969). This study was conducted to determine: (1) the uptake by and disposition of Cd in mosquitofish tissues and embryos; (2) the relationships between Cd accumulation and parasitism; and (3) chronic effects of Cd exposure and parasitism on survivorship, reproduction and growth of mosquitofish.

Materials and methods

Mosquitofish used in this study were seined from Asphalt Pond on the U. S. Department of Energy's Savannah River Plant, Aiken County, South Carolina. Fish from this pond had previously been determined to be free of helminth parasites. One half of the *G. affinis* were retained in a plastic lined wading pool (0.61 m high by 2.44 m diam) while the remaining fish were infected with metacercaria of the strigeid trematode (*Diplostomulum scheuringi*) by placing them in a screen enclosure (1 m^2 by ½ m deep) in Par Pond, a large cooling reservoir (TILLY 1975). Infections were acquired passively via direct penetration by cercaria released from the large natural population of the snail *Helisoma trivolvis*, which is the first intermediate host for the parasite. After four weeks of exposure, the enclosed fish were removed from Par Pond and placed in a retaining thank identical to that holding the uninfected *G. affinis*. Both groups of fish were retained an additional three weeks under identical conditions prior to cadmium exposure. Both groups of fish were fed ad libitum with Tetramin® tropical fish food at least three times a week.

Cadmium dosing was performed outdoors in ten separate flow-through systems. Controls and treatments of 1.15, 2.1, 3.7, and 6.5 μg Cd $\cdot$ l^{-1} were assigned randomly, with two replicates per treatment. Fifty-five gallon polyethylene-lined drums were used as header tanks. Drums were filled with conditioned well water, and Cd concentrations adjusted with an aqueous standard solution of $CdCl_2$. Each drum drained through a sediment trap to a covered 7 l polyethylene box which contained the fish. Flow rates through the boxes were adjusted to deliver approximately five volume changes per day. Header drums were refilled every three to four days and Cd concentrations adjusted volumetrically. The water used in this study was soft and acid (Table 1) which is typical of surface waters on the upper coastal plain of the southeastern U. S. A. During the study period the water temperature ranged from 22 °C in early October to approximately 1 °C when the experiment was terminated on 30 November.

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Table 1. Quality of treatment water.

Total alkalinity	17.5 $mg \cdot l^{-1}$ as $CaCO_3$
Hardness	21.5 $mg \cdot l^{-1}$ as $CaCO_3$
pH	6.6
Specific conductance	31 $\mu mho \cdot cm^{-2}$
Ionic strength	2.5×10^{-4} M
SO_4^{-2}	1.9 $mg \cdot l^{-1}$
Total P	10.1 $\mu g \cdot l^{-1}$
Nitrogen (NO_2 + NO_3)	19.2 $\mu g \cdot l^{-1}$
Ca	3.17 $mg \cdot l^{-1}$
Cu	3.4 $\mu g \cdot l^{-1}$
Co	2.5 $\mu g \cdot l^{-1}$
Cd	0.023 $\mu g \cdot l^{-1}$
Cr	0.3 $\mu g \cdot l^{-1}$
Fe	1.7 $\mu g \cdot l^{-1}$
K	1.1 $\mu g \cdot l^{-1}$
Mg	246 $\mu g \cdot l^{-1}$
Mn	7.0 $\mu g \cdot l^{-1}$
Na	1.8 $mg \cdot l^{-1}$

Once flow rates and Cd concentrations were adjusted, 10 infected or non-infected *G. affinis* were placed into polyethylene boxes. Each system was inspected daily, and dead fish removed and autopsied. The remaining fish were fed, water temperatures recorded, and flow rates adjusted. Water samples were acidified with 2% redistilled HNO_3 and frozen in 20 ml polyethylene bottles until analysed. Fish dying during the first 38 hours of the experiment were replaced by another individual of the same group (parasitized or non-parasitized). Thereafter, dead fish were not replaced during the eight-week experimental period. At the end of the eight-week exposure period, all remaining fish were frozen immediately and stored at −4 °C until Cd analyses could be performed. Each individual was sexed, measured and dissected, using stainless steel implements. All samples were dried over $CaSO_4$ and weighed with a model 4700 Cahn electrobalance.

Fish were wet ashed at 85 °C with 4 ml redistilled $HNO_3 \cdot g^{-1}$, dry weight, of tissue (Giesy & Wiener 1977) and then diluted to 5 ml. Parasites, fish embryos, and fish livers were wet ashed at 65 °C on 1 cm diameter platinum pans in 1 ml glass volumetric flasks. 40 µl of redistilled HNO_3 were added to each pan (sample) and the flasks suspended in a water bath. Each sample was subsequently diluted to 1 ml with double distilled water.

Cadmium concentrations were determined using a Perkin-Elmer model 306 atomic absorption spectrophotometer, which was equipped with an HGA-2100 flameless atomizer and deuterium continuum background corrector. Cadmium was stabilized, using $(NH_4)_2SO_4$ additions, so that matrix interferences could be eliminated by increasing pyrolysis temperatures (Briese & Giesy 1975). Reagent and technique blanks were used throughout preparation and analytical procedures to evaluate possible contamination. Sample preparation and analytical procedures were evaluated and validated by comparing results to U.S. National Bureau of Standards bovine liver as a reference material (Skinner et al. 1977). Possible matrix interferences were evaluated by standard additions and measurement at an adjacent, nonabsorbing wavelength. Reportable limits in tissues varied, due to varying tissue weights and tissue concentrations. Reportable limit (10 SD of background signal) was approximately 50 pg with an injection volume of 10 µl.

To simplify (initial) data analyses, we assumed an essentially unidirectional movement of Cd through the fish organs and the Cd concentration in any single compartment to be dependent primarily upon the concentration of Cd available from the preceding compartment (Fig. 1). We subsequently constructed stepwise multiple regressions utilizing the "proximal" Cd source as the first independent variable, and arrayed more "distal" sources in descending order of their contributions to the coefficient of determination (R^2) for the total regression. Cadmium concentrations of different compartments were not totally independent, and analysis of covariance was used in pairwise comparisons of independent variables to correct for covariate effects. Cadmium concentrations in tissues were log-normally distributed; thus, means and standard errors were calculated after log-

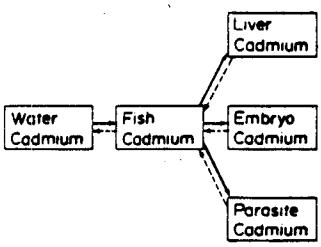

Fig. 1. Schematic concystualization of unidirectional flow model of Cd accumulation and transluation in *G. affinis* and associated embryos and parasites.

transforming the data and back-transforming before presentation (Giesy & Wiener 1977). Correlations of log-transformed and untransformed data were always very similar, and the acceptance or rejection of significance in correlations not affected. Pearson correlation coefficients or partial pairwise correlations were determined for each variable couplet. For this reason, the number of degrees of freedom varied among treatment combinations. Relationships between and among variables were deemed not significant if $P \geq 0.10$, while probabilities of significant correlations are listed. Statistical calculations were performed using the Statistical Analysis System "SAS" (Barr et al. 1979) and an IBM 360-195 computer.

Results

Neither Cd nor parasite main effects nor parasite-Cd interaction significantly affected mosquitofish growth, nor was Cd or parasite induced mortality observed. Mean recoverable liver weights were negatively related to liver Cd concentration ($R = 0.47$, $n = 143$, $P \leq 0.0001$).

Only nine broods of embryos were observed in 169 female mosquitofish which were dissected for analysis. All pregnant fish were non-parasitized ($\chi^2 = 11.28$, $df = 1$; $P < 0.002$). The number of broods born during the course of the experiment is not known. However, all embryos observed were fully developed and approaching parturition. Mean embryo dry weight of individual embryos was inversely proportional to brood size ($r = -0.779$, $n = 9$; $P = 0.013$). The number of embryos present was not significantly correlated with fish size. Cadmium concentrations in neither water, fish, nor

Table 2. Cadmium concentrations. Means and 95% CI were calculated from log-transformed data. The range and sample size are given for each mean.

	$\mu g\ Cd \cdot l^{-1}$				
Water – nominal	0.0	1.15	2.1	3.7	6.5
Water – analysed (mean ± t0.05 · SE)	0.02±0.1 n=4	2.3±0.3 n=4	3.1±0.2 n=4	5.6±0.5 n=4	7.5±0.2 n=4
	$\mu g\ Cd \cdot kg^{-1}$, dry weight				
Fish livers	12330.1 (9259–16420) n=28	15371.5 (12859–18375) n=37	12410.2 (10023–15366) n=36	21695.8 (18482–25468) n=36	20662.1 (15966–26739) n=26
Residual fish tissue (liver excluded)	853.7 (658–1107) n=31	1514.5 (1363–1683) n=39	1979.7 (1755–2234) n=31	2719.5 (2427–3048) n=37	3619.3 (3067–4272) n=38
Fish embryos	20.9 n=1	290.5 (74–1134) n=2	46.0 n=1	1302.2 (837–2026) n=5	–

Table 3. Pairwise PEARSON correlations (r) of tissue Cd concentrations, Tissue Cd vs. source Cd. Sample size (n) is in parentheses; probability of significance is given by symbol.

Tissue	Cadmium sources residual tissue	Water
Residual tissue	–	***
		0.69
		(180)
Liver	***	***
	0.36	0.30
	(157)	(159)
Embryo	**	*
	0.87	0.82
	(9)	(9)

* = $P \leq 0.01$, ** = $P \leq 0.005$, *** = $P \leq 0.0001$.

fish liver were significantly correlated with the number of embryos present. The number of embryos present was not significantly correlated with mean embryo Cd concentration.

Parasite burdens in infected fish varied from one to 36 flukes, and were proportional to fish size. The number of parasites was significantly correlated with liver weight (+0.32, $n = 76$, $P \leq 0.006$), but the correlation was not significant when corrected for the fish length covariate. There was no significant correlation between recoverable liver weight and mean weight of individual parasites. Neither water nor fish Cd concentration affected the number of parasites present when corrected for the fish length covariate. However, the mean dry weight of individual parasites was significantly and negatively correlated with Cd concentrations in fish ($r = -0.24$, $n = 91$, $P \leq 0.022$).

Fish weight was negatively correlated with liver Cd concentration ($r = -0.18$, $n = 157$, $P \leq 0.02$) and embryo Cd concentration ($r = -0.62$, $n = 9$, $P \leq 0.12$), but there was no significant correlation between fish weight and fish Cd body burden. Recoverable liver weight was significantly and negatively correlated with liver Cd concentration in both parasitized and non-parasitized fish (parasitized, $r = -0.45$, $P \leq 0.0001$, $n = 71$; unparasitized, $r = -0.42$, $P \leq 0.0005$, $n = 72$; parasitized and unparasitized pooled, $r = 0.42$, $P \leq 0.0001$, $n = 141$).

Cadmium concentrations of fish livers and residual fish tissues were proportional to water Cd concentrations (Tables 2—4). Cadmium accumulation by fish and fish livers was not affected by parasite burden. Consequently, parasitized and non-parasitized fish were pooled for further statistical analyses. The simple, unidirectional flow model proved satisfactory for analysing Cd partitioning in mosquitofish. In each compartment, Cd concentrations were most highly correlated with that in the preceding compartment of the model (Tables 2—4, Fig. 1). Stepwise, multiple regressions revealed that once the Cd content of the preceding compartment was included in the model, addition of further Cd sources did not contribute significantly ($P < 0.05$) to the regression. Predictive equations using a single independent variable accounted for approximately 50—60% of the total variation in Cd concentrations (Table 3) but were not very accurate in predicting Cd concentrations in individual fish. Cadmium concentrations in embryos were directly proportional to the Cd content of whole fish, fish liver, and water (Tables 2 and 3), but were much lower than those observed in fish livers and residual tissues.

Table 4. Linear regressiones of the form Y = a X + b relating Cd concentrations in embryos, fish livers and residual fish tissues (dry weight basis).

Log residual fish Cd (ppm)	= 3.03+0.09 water concentration (ppb) (r = 0.87)
Fish liver Cd (ppm)	= 1.27×10^{4} + 2.87 residual fish Cd (ppm) (r = 0.70)
Embryo Cd (ppm)	= -8.3×10^{2} + 0.69 residual fish Cd (ppm) (r = 0.80)

Discussion

The Cd exposure concentrations in this study were very low, relative to the acute toxicity of Cd to *G. affinis* (96 h-LC_{50} = 2.2 mg Cd · l^{-1}, Giesy et al. 1977). At the time the study was conducted, the EPA water quality standard was 10 μg Cd · l^{-1}. This has subsequently been lowered to 0.4 μg Cd · l^{-1} for sensitive organisms in soft water (EPA 1976), such as that used in this study. Applying an application factor of 1/100 of the 96 h-LC_{50} suggested by Waller et al. (1976) would give safe ranges of approximately 20 μg Cd · l^{-1}. Thus, we chose the chronic exposure concentrations used in this study to examine the chronic effects of Cd exposure on reproduction.

Cadmium uptake by *G. affinis* is primarily via direct absorption from the water, rather than from the diet (Williams & Giesy 1978). Size of fish and surface/volume ratios might by expected to influence Cd accumulation, but metabolic and feeding rates have minimal effects. Uptake across gill membranes may be enhanced by increased metabolism, due to parasite burden, with a concomitant increase in gill ventilation rate. Parasitism often affects the metabolism or feeding rates of the host (Kennedy 1972) and may influence the uptake of those pollutants acquired via ingestion.

Cadmium concentrations of fish embryos were very low, compared to maternal fish tissues. In poeciliid fish, such as *G. affinis*, the ovarian, vitelline and embryonic membranes may provide a barrier to Cd. Recent studies have shown that the mammalian placenta effectively limits the uptake of Cd by embryos (Baglan et al. 1974; Ahokas et al. 1976). A study of female zebra fish exposed to Cd for six months showed that Cd is retained by the adult and not transferred to the offspring (Rehwoldt & Karimian-Teherani 1976).

The fact that all pregnant fish were unparasitized indicates a significant interaction between Cd exposure and parasite burden, even though we only observed nine broods. In local field populations which are heavily parasitized with *D. scheuringi*, female *G. affinis* are able to continue normal reproduction. Also, in this study Cd exposure alone did not prevent the development of embryos. At the end of the study, all embryos were fully developed and approaching parturition, which suggests that no pregnancies were initiated during the period of Cd exposure, even though mature males were available. Exposure of parasitized fish to cadmium may have caused abortion of pregnancies.

Infection of *G. affinis* by *D. scheuringi* is passive on the part of the host. Acquisition rate of the flukes is a function of the density of the infective cercarial stage in the water and of the size of the host. Since all infected *G. affinis* were exposed under the same conditions for the same length of time, the distribution of flukes among the fish was a random variable (Poisson distribution) with the mean determined by the probability of encounter between the two organisms. This was in turn a function of the size of the fish.

Across treatment statistical comparison of the number of parasites relative to the size of *G. affinis* used in this study revealed no significant differences. We consequently concluded that subsequent exposure to Cd had no effect upon the numbers of parasites surviving in the fish.

Parasite size, however, was negatively correlated with Cd concentrations in the host. This may have resulted from a decrease in growth rate or from a Cd induced limit on attainable size. In an earlier study at ambient temperatures of 20 °C and above, *D. scheuringi* in *G. affinis* were still growing after 60 days. It is unlikely that the fluskes reached maximum size in 66 days under the lower ambient temperatures reported here. Suppression of growth rates by Cd has been reported previously in bluegill sunfish (Cearley & Coleman 1974; Benoit et al. 1976; Eaton et al. 1978). Only mature *G. affinis* were used in this study, and growth rates at the temperatures experienced here prevented our determining whether similar growth suppression occurred in the host. The reduced growth of parasites may also have been the result of indirect effects such as decreased available energy in the host, reduced vitamin B_{12} concentrations (Merlini 1978), alteration of liver enzymes, which are known to be affected by Cd (Jackim et al. 1970) or an increase in nonspecific resistance to stressors as expressed by the general adaptation theory (Selye 1976). The livers of *G. affinis* exposed to greater Cd concentrations were more diffuse and less easily isolated for analysis, and mean dry weights of recoverable liver tissue were significantly lower at higher Cd exposure concentrations. Therefore, Cd could have reduced parasite size by direct effects on the parasites or indirectly due to effects on fish liver tissue.

When determining the effects of drugs or toxicants, several factors such as age, sex, genotype, nutritional state, and present or past diseases result in considerable variability (Toothill 1977). Parasites increase the toxicity of Zn to the three spined stickleback (Pascoe & Cram 1977) and sockeye salmon (Boyce & Ymada 1977). This reduction in resistance to metal stressors by parasites has been explained by a general weakening of the host organism due to an increased energy demand due to the parasitism. Esch et al. (1975) discuss parasitism in terms of general adaptation syndrome and the interactions of stressors and parasite burdens in aquatic animals.

While we did not observe any synergistic effects on survivorship of adult *G. affinis*, we did find that the weight of adult *G. affinis* was inversely correlated with the Cd concentrations in the liver. Mean parasite weight was also inversely proportioned to Cd concentration in the liver. The most striking result was the fact that no fish which were exposed to Cd and parasitized were pregnant. In field populations which were heavily parasitized with *D. scheuringi*, female *G. affinis* were able to continue to reproduce. Therefore, the interactions of Cd exposure and parasitization completely blocked reproduction. Our results support the contention that there is a synergistic effect of Cd and parasitization on reproduction.

Summary

Non-parasitized mosquitofish and mosquitofish parasitized with metacercaria of *Diplostomulum scheuringi* (Trematoda: Strigeidae) were exposed for 55 days to 0.02, 2.3, 3.1, 5.6, and 7.5 $\mu g\ Cd \cdot l^{-1}$ added as $CdCl_2$ in a continuous flow system. Cadmium accumulation by whole fish and fish livers was proportional to water Cd concentrations. Mean whole fish and fish liver Cd concentrations across treatments ranged from 1.0 to 4.1 and 15.6 to 24.0 $\mu g\ Cd \cdot g^{-1}$, dry weight, respectively. Cadmium accumulation by fish was not affected by parasite burden. Cadmium concentrations in

embryos were directly proportional to Cd content of whole fish, fish livers, and water. Parasite numbers were proportional to fish size and were not affected by Cd concentrations in water or fish. Mean parasite weight was inversely proportional to fish whole body Cd concentrations. No Cd- or parasite-induced effects were observed on mortality or growth of mosquitofish. Fish embryos were observed only in non-parasitized individuals. Mean embryo weight was inversely proportional to brood size. Mean recoverable liver weight was negatively related to both whole fish and liver Cd concentrations.

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Authors' addresses:

J. P. GIESY, Pesticide Research Center and Department of Fisheries and Wildlife, Michigan State University, East Lansing, Michigan 48824, U. S. A.

D. H. SMITH, Instituto de Biologia VFBA, Rua Barad de Gereboabo, Salvador, Brazil