Developments in Environmental Science, Volume 5
D. Sarkar, R. Datta and R. Hannigan (Editors)

ISSN: 1474-8177/DOI:10.1016/S1474-8177(07)05023-1

Chapter 23

Linkages between diet and metal accumulation in crayfish

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Abstract

Crayfish plays an important role in the environmental biogeochemical cycling of metals in terrestrial aquatic ecosystems by processing sediments, organic matter, algae, and phytoplankton, as well as consuming detritus, carrion, and various fish species.

Although their importance in biogeochemical cycling has been long established little is known of the fractionation of metals from food source to tissue. Understanding of elemental behavior at this level therefore provides critical insight into the role of sediment processors in the biogeochemical cycling of metals. Crayfish can potentially store metals in both soft and hard tissue. This has consequences for bioaccumulation. For example, if crayfish shed most accumulated metals through molting, and the exoskeleton is buried or otherwise left undisturbed, one can consider the exoskeleton a net sink for metals. To explore the partitioning of metals in crayfish tissue, crayfish were collected at the Spring River in Hardy, AR. Treatment groups were selected with each being fed a diet consisting of known concentrations of metals as well as two control groups which were fed standard, non-spiked diets. Metal concentrations in the gills, muscle, and exoskeleton over the duration of the experiment were analyzed by DRC-ICP-MS. In the case of tissues there is a consistent and predictable relation between diet and tissue chemistry. Certain metals such as Cd and Pb were found in the exoskeleton in amounts proportional to that of the diet. Concentrations in the exoskeleton increased over time as expected. Our data suggests that biomagnification of metals in crayfish tissue is directly related to environmental concentrations with crayfish acting as passive "processors" rather than "fractionators" of metals.

23.1. Introduction

The objectives of this study are to identify what metals are taken up by crayfish and determine if they are stored or excreted, determine if the metals are taken in through the gills or the diet, and identify if crayfish can be used to biomonitor metals. It is hypothesized that diet is a pathway for metal storage in the crayfish tissues and through a metal-spiked diet, crayfish will store and accumulate some metals.

The crayfish is an invertebrate, common to many rivers, streams, lakes, ponds, and floodplains throughout North America (Hobbs, 1989). Crayfish occupy a wide range of habitats because of ecological, behavioral, and physiological adaptations through nearly 300 million years of evolution (Holdich and Lowery, 1988; Hasiotis and Mitchell, 1993; Hasiotis, 1999). Most species of crayfish are able to survive equally well in both moving and standing water bodies provided there are suitable physical, biological, and chemical conditions. Some have the ability also to colonize aquatic systems with such extremes in water quality as varying pH and temperature (Holdich and Lowery, 1988). Crayfish have the ability to accumulate high concentrations of toxic metals without experiencing unfavorable effects (Geisy et al., 1980).

Some species of crayfish have the ability to regulate such physiologically important metals as Zn (Mirenda, 1986) and Cu (Anderson, 1978). Other metals like Cd and Pb, which are not physiologically important, have been shown to accumulate passively in crayfish in proportions similar to those in their environments (Anderson, 1978). Regardless of the mechanism of uptake, active or passive, crayfish play an important role in the distribution of metals in the food web of aquatic environments by feeding on algae and organic matter, processing sediment and serving as a major prey species for many kinds of fish. For this reason, if metals are accumulated in crayfish through diet or by intake through the gills, they could be accumulated subsequently in fish and other vertebrates higher up in the food chain or effectively immobilized by storage in the molt. By interacting with sediments, crayfish may also play an important role in the redistribution of metals. Periphyton and phytoplankton, upon which crayfish feed, are known to participate in exchange processes of metals. For example, high concentrations of lead, zinc, and copper were found in algae samples from a metal-polluted Missouri stream (Hasset et al., 1980). Periphyton and phytoplankton can therefore serve as a source of metals in crayfish (Hart, 1982).

Studies show that crayfish accumulate some metals such as Cd and Pb based on relative concentrations in the environment (Anderson, 1978) and also through increased exposure to low pH waters (Young and

Harvey, 1990; DiStefano and Neves, 1991). Crayfish from river sites with high Cd and Pb inputs were found to have high levels of the metals (2.22 ppm Cu and 27.39 ppm Pb per dry weight of crayfish). If crayfish muscle, gills, and exoskeleton are sinks for certain metals it may be possible to use their chemistry for bio-monitoring of those metals in the aquatic ecosystem.

23.2. Methods

The experimental conditions consisted of eight 10-gallon aquaria tanks with two of these tanks serving as controls and the other six as treatments. Six crayfish were placed in each control and treatment. Six pieces (7.62 cm each) of PVC pipe were placed in each tank to serve as hiding areas for the crayfish and to minimize carnivorous behavior.

The metal-enriched food was prepared using Wardley shrimp pellets. The pellets were powdered using a Zircon mill. 283.5 g (10 ounces) of the shrimp meal was used for each treatment, with the two control groups receiving an unaltered diet. Using the prepared stock solution (Table 23.1), aliquots, along with the shrimp meal, were added to 500 ml of milli-Q water (18.2 Mohm) to obtain the desired concentrations given in Table 23.1. All of the metal standards were metal salts. Upon mixing there is no assumption that these elemental metals were speciated to a more bioavailable form. Rather ingestion of dilute metals salts, inorganic species, and associated accumulation of the metals can be attributed to diet and metabolic transformation of non-bioavailable metals. After thorough homogenization, a 60 cc flushing syringe was used to re-pellet the mixture onto trays that were then heated at 250°C for 2 h to dry out the pellets. While the crayfish food (excluding control) was metal spiked in increasing concentrations, the sub-samples taken do not reflect these increments.

Water samples were collected every two months over the course of the study for a total of four samples per tank. Food samples were taken before and after the duration of the experiment and analyzed for metal

Table 23.1. Concentrations of metals in each treatment in $\mu g\, g^{-1}$. Standards for spiking of shrimp pellets were made from the metal salts as shown

Formula	Stock	T1	T2	T3	T4	T5	T6
$HgCl_2$	2715	271.5	27.15	2.715	0.2715	0.02715	0.002715
$Cd(CH_3CO_2)_2 \cdot 2H_2O$	26653.4	2665.34	266.534	26.6534	2.66534	0.266534	0.026653
$Pb(NO_3)_2$	33120	3312	331.2	33.12	3.312	0.3312	0.03312

content. The lack of homogeneity of the food samples could be a limitation to the experimental design because unless all the food is consumed, the crayfish do not receive the total intended dose of metals.

A single species of crayfish (*Cambarus hubbsi)*, collected from the Spring River at Hardy, Arkansas, were fed a metal-enriched diet for an eighth month period from early August 2003 to end of March 2004. Molts and carcasses of dead crayfish were collected 2–3 times a week and frozen for subsequent analysis. Although the hepatopancreas is an important organ for food digestion and waste processing, it was not sampled. In this study we were only concerned with metal storage in the soft tissues and the exoskeleton from the diet, not in the digestive and physiological organs.

Upon termination of the study, remaining crayfish were euthanized and frozen. Crayfish were dissected and the gill, muscle tissue, claw, and exoskeleton separated. All crayfish parts and molts were placed, separately, into 50 ml Teflon tubes. 1 ml of ultra-pure nitric acid and 1 ml of 35% hydrogen peroxide were added to each tube. Weights of crayfish parts varied from 10–1000 mg and the reported concentrations are weight corrected. Watch glasses were placed on the top of each tube and then placed on a digestion block at 80°C for about 3 h. Upon complete digestion, the watch glasses were removed and the samples were dried to a cake in the digestion block. After drying, the samples were brought into a solution by adding 0.5 ml of ultra-pure nitric acid along with 2 ml of an internal 1000 ppb In standard. Final internal standard concentration was 40 ppb. The samples were then brought to 50 ml volume in volumetric tubes using 18.2 Mohm water.

Concentrations of Cd, Hg, and Pb were measured by inductively coupled plasma mass spectrometry (PerkinElmer ELAN 9000) following EPA 6020. Concentrations were calculated based on least squares regression of five elemental calibration standards ($0.1\,ng\,g^{-1}$ to $1\,\mu g\,g^{-1}$, spiked with $40\,ng\,g^{-1}$ Indium). Quality control was measured by two

Table 23.2. Average treatment concentrations ($\mu g\,g^{-1}$) for whole body total metal content

	Cd	Hg	Pb
T1	365.5953	2395.846	383.3205
T2	203.7375	531.6307	161.7169
T3	165.9837	360.6048	96.01894
T4	67.84018	15.04965	86.31192
T5	48.84657	437.3458	93.20399
T6	383.998	148.8616	266.9582
C1	85.54701	1.587691	42.50354

Table 23.3. Average treatment concentrations ($\mu g\,g^{-1}$) for exoskeleton

	Cd	Pb	Hg
T1	34.61539	154.8646	747.3635
T2	218.9685	239.4492	708.596
T3	42.21743	63.3588	46.80132
T4	134.9583	170.366	44.05
T5	106.5266	138.9873	1092.944
T6	250.4659	278.2298	1.772144
C1	69.15562	16.29173	1.764735

Table 23.4. Average treatment concentrations ($\mu g\,g^{-1}$) for tissue

	Cd	Pb	Hg
T1	606.5466	568.5739	3894.128
T2	106.7864	0.756923	
T3	44.35711	140.0057	607.2782
T5	0.574571	0.932901	0.273001
T6	542.5269	283.2979	259.3928
C1	97.84055	62.1624	1.469662

quality control standards run as unknowns ($5\,ng\,g^{-1}$, $150\,ng\,g^{-1}$) with precision better than 95%. Relation between weight-corrected concentration data was explored using ANOVA, parametric and non-parametric bivariate correlations, and linear regression (InStat 4.0). For the purpose of data analysis, the carapace and claws were grouped together as "exoskeleton", with the gills and muscle tissue grouped as "tissue". Mercury, cadmium, and lead are reported in $\mu g\,g^{-1}$ (ppb) (Tables 23.2, 23.3, 23.4).

23.3. Results

Figure 23.1 shows results of the treatment concentration versus whole body total Hg (sum of all tissues). Control and treatment groups are abbreviated with C and T respectively. It is important to note that because the crayfish did not consume all of the metal-enriched food, the relationships presented here can only be considered as loose generalizations or as initiations of a trend. As can be seen from this figure, there appears to be a relationship between food Hg concentration and Hg content in the whole crayfish although this relation is not statistically significant. This trend was also recorded by the soft tissue (Fig. 23.2) but not by the exoskeleton (Fig. 23.3). Overall, there was a moderate

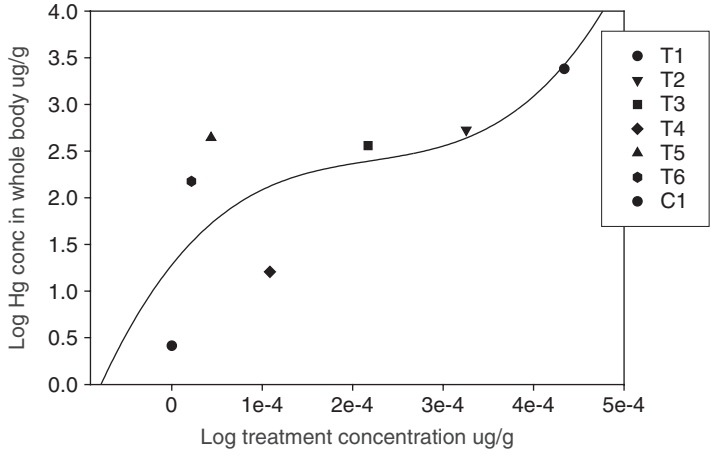

Figure 23.1. Mercury concentration ($\mu g\, g^{-1}$) of the whole crayfish compared to the concentration of individual treatments. Highest concentration treatment on far right with control group on the far left.

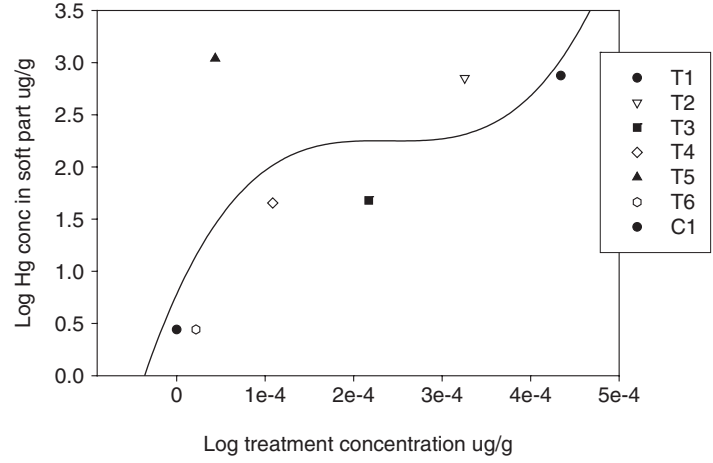

Figure 23.2. Mercury concentration of the crayfish tissue compared to individual treatment groups.

treatment effect however not statistically significant at an alpha of 0.05. Although neither regression analysis nor ANOVA substantiated the trend suggested by these results, further investigation of these trends may elucidate this relation more discretely. Figure 23.4 shows results of the treatment concentration versus whole body total Cd. As can be seen from

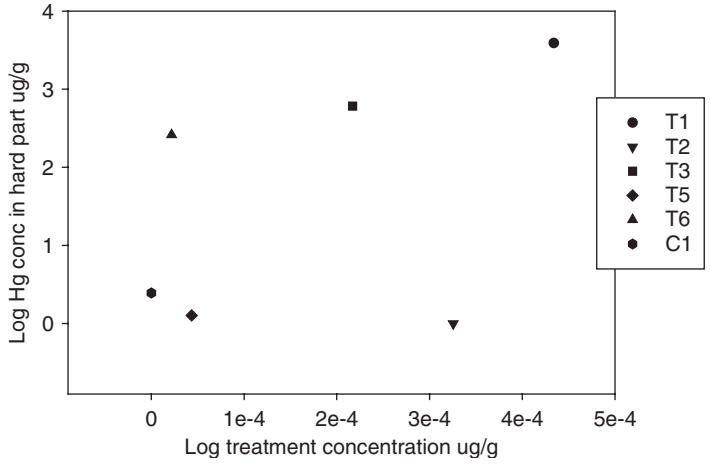

Figure 23.3. Mercury concentration of the crayfish exoskeleton compared to individual treatment groups. Treatment 4 failed to pass QA/QC (EPA 6020) and so no data are presented here.

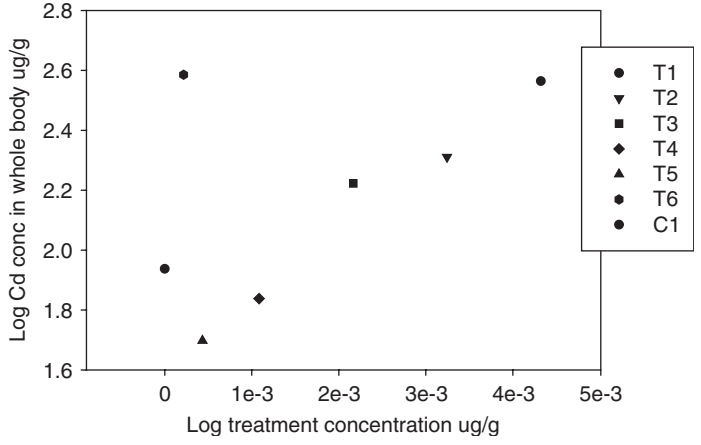

Figure 23.4. Cadmium concentration ($\mu g\, g^{-1}$) of the whole crayfish compared to the concentration of individual treatments.

this figure, there is a relationship between food Cd concentration and Cd content in the whole crayfish. This trend was not recorded by the soft tissue (Fig. 23.5) or the exoskeleton (Fig. 23.6). Data points for treatments 5 and 6 are off-scale compared to the higher concentrated treatments in all figures for Cd. This could be due to a threshold concentration

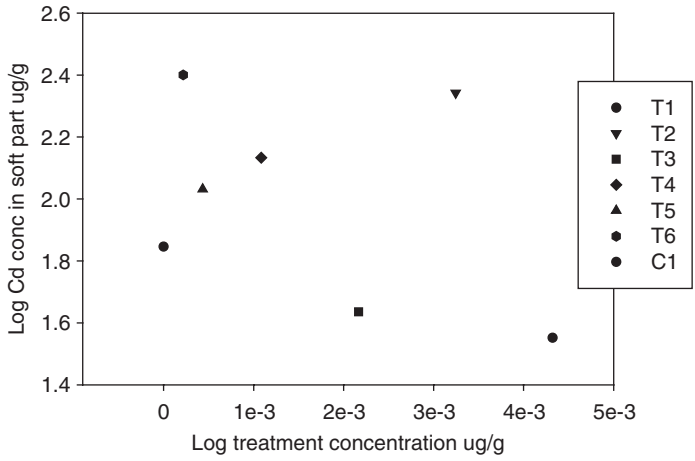

Figure 23.5. Cadmium concentration of the crayfish tissue compared to individual treatment groups.

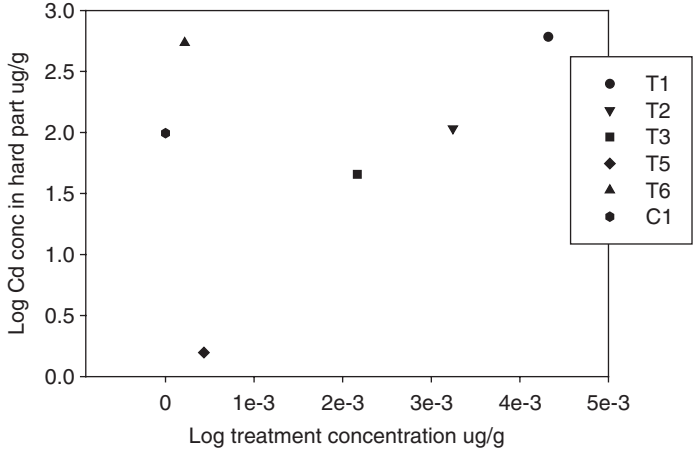

Figure 23.6. Cadmium concentration of the crayfish exoskeleton compared to individual treatment groups. Treatment 4 failed to pass QA/QC (EPA 6020) and so no data are presented here.

below which it is difficult to resolve interferences. Overall, there is only a significant treatment difference in the whole body total Cd treatments when compared to the control. No treatment effect was found for Lead. As can be seen in Fig. 23.7, there seems to be a treatment effect for the first three treatments but the data means are nearly identical.

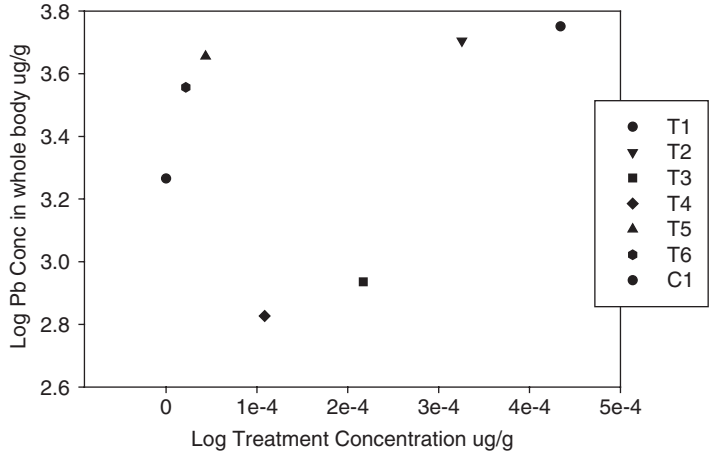

Figure 23.7. Lead concentration of the crayfish exoskeleton ($\mu g\, g^{-1}$) compared to individual treatment groups.

23.4. Discussion

Trace elements enter and leave a stream in the form of soluble, colloidal, and particulate matter. Crayfish interact and assist with the cycling of these forms of matter by residing in or near the water column where water is continually flushed over the gills, and by serving as detritivores, herbivores, carnivores, and scavengers. For example, crayfish can process several types of metal complexes (soluble, colloidal, and particulate matter) associated with the biota (phytoplankton, periphyton, and bacteria) upon which they feed. As carnivores and scavengers, crayfish are susceptible to metal uptake by predating on fish and carrion. Some freshwater fish, whose diet often includes crayfish, are known to accumulate methyl mercury and may be a sink for some other metals as well (Gilmour et al., 1991). As detritivores, crayfish assist in the breakdown of organic matter (both coarse particulate organic matter and fine particulate organic matter). Natural organic matter has also been shown to aid in the cycling of metals by complexing the metals and keeping them in solution. Dissolved and colloidal organometals can then be washed over the gills, crossing the gill–blood barrier being accumulated in crayfish tissue. It is deduced that because crayfish interact with a variety of metals in most river systems, some of these metals are being stored in the soft tissue where they are not lost during the molting process.

When mercury enters an aquatic system, it can be converted by sediment residing bacteria to MeHg, a more toxic form that is

bioaccumulated up the food chain (Gilmour et al., 1991). It is well known that mercury, in the form of methyl mercury, is accumulated and stored in the tissues of many aquatic organisms (Campbell et al., 2003), and Hg has been found to accumulate in detoxifying organs (Wright and Welbourne, 1993; Mason et al., 2000). It is therefore logical to expect bioaccumulation of mercury in crayfish. The introduction of mercury into the food chain by crayfish can provide a potential pathway for exposure to humans due to crayfish being a major food source for many types of sport fish (Roel and Orth, 1993) which are consumed by humans. When the fish consume the MeHg-contaminated crayfish, the MeHg is transferred to the soft tissue in the fish where it is available to be accumulated by humans upon consumption. Understanding accumulation of mercury in crayfish from their diet is important because thousands of tons of mercury are released annually from power plants and incinerators, as well as from natural geological processes that make its way into the food web (Baker, 1998). This means that crayfish in many aquatic environments have readily accessible sources of Hg. Crayfish have been shown to be good monitors of heavy metal input into aquatic ecosystems (Vermeer, 1972), and results from this study indicate that crayfish may be good indicators of mercury inputs.

A treatment effect (increasing Hg in crayfish as Hg concentration increased in food) was observed for mercury for the whole crayfish and for the crayfish tissue. No treatment effect was found for the crayfish exoskeleton. There was a treatment effect for mercury storage in the soft tissue however. The mercury species used in this study was $HgCl_2$ which is a form that is 15–20% bioavailable.

Crayfish also accumulate Cd and Pb. Dickson et al. (1979) have shown that longer-ived species of crayfish accumulate more Cd and Pb in the tissue than shorter-lived species. Alikahn et al. (1990) found that the crayfish, *Cambarus bartoni*, from three lakes near a Sudbury smelter in Northwestern Ontario, Canada, had high concentrations of Cd in the hepatopancreas and gut of the crayfish. These concentrations varied with distance from the smelter with those closest to the site having the highest Cd concentration. Mwangi and Alikhan (1993) found high Cd concentrations in crayfish hepatopancreas after 3 days of lab exposure to Cadmium (12.5, 62.5, and 125 $\mu g\,l^{-1}$) but found no significant concentrations in the soft tissues when compared to the control.

Our results show an overall trend of increasing Cd in the whole crayfish with increasing food content. Unlike the whole crayfish, which revealed a treatment effect, the tissue and the crayfish exoskeleton did not reveal a treatment effect, indicating that there may be another tissue type or organ, such as the hepatopancreas, responsible for this trend.

No treatment effect for the tissue or exoskeleton was found for Pb in our data but other researchers have found significant cases of metal storage by crayfish. For example, adult crayfish exoskeleton and muscle tissue have been used to monitor concentrations of Pb and Cd in aquatic environments (Khan et al., 1995). In a study designed to monitor heavy metals in Tuskegee Lake (TL) and National Forest Creek (NFC), in Tuskegee, Alabama, Khan et al. (1995) found significant concentrations of Pb in the TL crayfish exoskeleton which was attributed to environmental contamination at the sample site. Naqvi et al. (1993) discovered that both Cd and Pb are readily accumulated in field-collected crayfish, *Procambarus clarkii*, although no significant biomagnification occurred from the main food source, alligator weed, to the crayfish. Anderson et al. (1997) found increasing Pb concentrations in the gills, muscle tissue, and exoskeleton of crayfish (*Procambarus clarkii*) after seven weeks of laboratory exposure to lead nitrate. Overall our results suggest that shedding of the exoskeleton is one possible mechanism by which crayfish void their system of metals. It is entirely possible that in addition to shedding that bodily waste may contain significant amounts of metals; however, we did not test the metal content of fecal material.

The results of this study show that Hg and Cd accumulated in the body of crayfish through the diet. Only a moderate correlation was established between metal accumulation and the spiked diet. This could be due to the fact that such key organs for metal storage as the hepatopancreas and gut were not sampled. In addition, it was not clear until the end of the study the degree to which homogenization of food would affect the results. Beyond grinding the food, no attempt was made to homogenize the food but in a controlled environment with only one food source, this is particularly important. While other researchers have found significant metal concentrations in crayfish in field conducted studies, where many forms of metal species were available, in the lab, where only one species of each metal was available, these results were not entirely duplicated.

ACKNOWLEDGMENTS

We thank Dr. Nate Bickford for helping MH initiate this project and Sarah Webb and David Clarke for all their help with sample preparation. We are especially grateful to the thorough reviews of the initial manuscript provided by Dr. Stephen Hasiotis and Dr. JoAnn Holloway. Funding for this research was provided by the Water-Rock-Life contract laboratory. Musical inspiration provided by Bob Dylan.

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