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Concentration Trends for Lead and Calcium-Normalized Lead in Fish Fillets from the Big River, a Mining-Contaminated Stream in Southeastern Missouri USA

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Abstract Lead (Pb) and calcium (Ca) concentrations were measured in fillet samples of longear sunfish (*Lepomis megalotis*) and redhorse suckers (*Moxostoma* spp.) collected in 2005–2012 from the Big River, which drains a historical mining area in southeastern Missouri and where a consumption advisory is in effect due to elevated Pb concentrations in fish. Lead tends to accumulate in Ca-rich tissues such as bone and scale. Concentrations of Pb in fish muscle are typically low, but can become elevated in fillets from Pb-contaminated sites depending in part on how much bone, scale, and skin is included in the sample. We used analysis-of-covariance to normalize Pb concentration to the geometric mean Ca concentration (415 ug/g wet weight, ww), which reduced variation between taxa, sites, and years, as was the number of samples that exceeded Missouri consumption advisory threshold (300 ng/g ww). Concentrations of Pb in 2005–2012 were lower than in the past, especially after Ca-normalization, but the consumption advisory is still warranted because concentrations were >300 ng/g ww in samples of both taxa from contaminated sites. For monitoring purposes, a simple linear regression model is proposed for estimating Ca-normalized Pb concentrations in fillets from Pb:Ca molar ratios as a way of

reducing the effects of differing preparation methods on fillet Pb variation.

Keywords Lead · Calcium · Mining · Longear sunfish · Redhorse suckers

The Big River (BR) and Flat River (FR), a tributary of the BR, drain the Old Lead Belt (OLB), a historical lead (Pb) and zinc mining area in Southeast Missouri USA (Fig. 1). About 180 km (81 %) of the BR, from the upper end of the OLB downstream to its confluence with the Meramec River (MR), and most of the FR have been contaminated by Pb and other metals. The Big River Mine Tailings/St. Joe Minerals Site was added to the U.S. Environmental Protection Agency (USEPA) National Priorities List in 1992 (USEPA 2016a), and much of the FR and BR have been designated as “Impaired” due to elevated concentrations of Pb and other metals in sediments and fish (Missouri Department of Natural Resources 2010). There has been a “Do Not Consume” advisory due to elevated Pb in suckers (Catostomidae) since 1980 (Czarnecki 1985) that has been expanded to include longear sunfish (*Lepomis megalotis*; sunfish) and several other species [Missouri Department of Health and Senior Services (MDHSS) 2016]. Fillet Pb concentrations in these species often exceed 300 ng/g wet weight (ww), the concentration at which MDHSS may issue a consumption advisory when considered together with other factors and exposure pathways [U.S. Environmental Protection Agency (USEPA) 2016b]. Reduction of mine waste inputs to the BR and FR has been a focus of environmental remediation in the OLB and contaminated sediment removal is being considered (USEPA 2016a). One long-term goal of remediation is to reduce Pb concentrations in fish and rescind the consumption advisory.

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Fig. 1 Fish collection sites on the Big, Flat, and Meramec rivers in Missouri (OLB Old Lead Belt)

Much of the Pb in the parts of fish that people typically eat occurs as a trace contaminant of calcium (Ca; Settle and Patterson 1980). As such, Pb tends to accumulate in Ca-rich tissues such as bone and scale. Mucus is also rich in Ca, can bind waterborne metals, and may represent a pathway for metal excretion (Varanasi and Markey 1978). Muscle Pb concentrations are usually low, but previous studies have shown that the inclusion of bone or scale, contact with mucus, or external contamination during sample processing or analysis can substantially influence measured Pb concentrations and increase sample-to-sample variability (Settle and Patterson 1980; Wiener 1982; Schmitt and Finger 1987; Schmitt et al. 2007). A comparison of “clean” processed (scaled, de-boned, skinless; prepared in clean-room) and “normally” processed (scaled, skin-on, not de-boned; prepared streamside) fillets from BR fish showed that Pb concentrations in fillet samples from

contaminated sites depend on how much material other than muscle is present, and that accounting for Ca can reduce the variance of fillet Pb by more than fourfold (Schmitt and Finger 1987).

Concentrations of Pb in BR fish have been monitored since the early 1980s (Czarnecki 1985; Schmitt and Finger 1987; Gale et al. 2004). However, determining whether fillet Pb concentrations have changed is difficult because fish collection and sample preparation procedures have differed among investigators and over time and Ca has not always been measured. Our objectives were therefore to determine whether Pb concentrations in BR fish have changed relative to historical concentrations after accounting for Ca, and to establish a baseline for future monitoring with a uniform protocol that includes Ca normalization.

Materials and Methods

Fillet samples were collected as part of a statewide monitoring program. The number and locations of sites sampled each year vary, and composite samples are analyzed as a cost-saving measure. Fish for this study were collected by electrofishing in July–September 2005 and 2010–2012 at sites located on the BR upstream and within or downstream of the OLB, on the FR, and on the MR upstream and downstream from the confluence of the BR (Fig. 1; Table 1). The sampling objective was three 5-fish composites of sunfish and either black redbreast (*Moxostoma duquesnii*) or golden redbreast (*M. erythrum*) at each site visit. Fish were grouped by species, measured (total length, mm), weighed (g), and sorted into 5-fish composite samples in which the smallest fish was at least 75 % as long as the largest [U.S. Environmental Protection Agency (USEPA) 2000]. Fish were killed by cervical dislocation, after which a skinless fillet was obtained from one side of each fish from the ribs to the caudal peduncle. The fillet was rinsed in distilled water and placed on aluminum foil. The five fillets constituting each composite sample were wrapped together in foil, placed in a labeled plastic bag, and stored on ice until transported to freezer for storage at -20°C . Workspace and equipment were replaced or rinsed with distilled water and isopropyl alcohol between each composite sample.

In the laboratory the frozen composite samples were partly thawed, chopped and minced, and freeze-dried. Moisture content was determined gravimetrically. The dried samples were pulverized to a fine powder, of which 0.25 g were digested with 6 mL of nitric acid and analyzed for Pb and Ca by inductively coupled plasma mass

Table 1 Fish collection sites on the Big (B), Flat (F), and Meramec (M) rivers, 2005–2012 (OLB Old Lead Belt)

River and site name	Site description	River km	UTM X ^a	UTM Y ^a	Year(s) sampled
<i>Meramec</i>					
Greentree Park	Below Big R.	28.5 ^b	722377	4270844	2010
Allenton Access	Above Big R.	5.5 ^b	704117	4261073	2011, 2012
Rte. 66 State Park	Below Big R.	7.2 ^c	708141	4265966	2011, 2012
<i>Big</i>					
House Springs	Below OLB	16.1 ^d	710245	4254900	2011, 2012
Mammoth Access	Below OLB	101 ^d	703747	4221830	2005, 2010, 2012
Cherokee Landing	OLB	145 ^d	714959	4203610	2010, 2012
Flat R. above Big R.	OLB	0.8 ^c	719928	4196240	2012
Desloge (Hwy. 67)	OLB	166 ^d	719734	4196715	2012
Irondale (Hwy. U)	Above OLB	204 ^d	703224	4189516	2010, 2012

^a Universal Transverse Mercator (Zone 15) easting (X) and northing (Y), World Geodetic System 1984 datum

^b Below Big R. confluence

^c Above Big R. confluence

^d Above Meramec R. confluence

spectrometry; Ca was analyzed as indicator of material other than muscle in the samples (Schmitt and Finger 1987; Schmitt et al. 2007, 2009). Analytical results in ng/g (Pb) or µg/g (Ca) dry weight (dw) were converted to ww using each sample's moisture content.

Quality assurance (QA) measures included the analysis of certified reference materials, blanks, replicates, and fortified samples. Method detection limits were 1.6–2.9 µg/g dw for Ca and 7.0–9.0 ng/g dw for Pb, with method quantitation limits (MQLs) of 5.3–9.6 µg/g dw and 23.0–30.0 ng/g dw, respectively. Concentrations in all samples exceeded MQLs and all quality-control results were considered acceptable. Five samples (2 redhorse, 3 sunfish) were analyzed in triplicate as part of the QA; relative standard deviations were 17 %–26 % for Ca and 26 %–60 % for Pb, which are typical for these heterogeneously distributed elements. Including the five samples analyzed in triplicate, the dataset comprised 102 observations representing 92 composite fillet samples (14 black redhorse, 32 golden redhorse, and 46 sunfish). Raw data and details of the laboratory procedures and QA are available elsewhere (<http://dx.doi.org/10.5066/F7125QR2>; May et al. 2013).

Version 9.3 of the Statistical Analysis System (SAS Institute, Cary, NC USA) was used for statistical analyses. All data were log₁₀-transformed. The molar ratio of Pb to Ca ([Pb]/[Ca]) was computed from measured concentrations and consensus atomic weights (Pb 207.19, Ca 40.08) × 10⁶. Concentrations of Pb and Ca in black and golden redhorse from the BR tend to be similar (Czarnecki 1985; Schmitt and Finger 1987), so these species were considered equivalent (“redhorse”). Preliminary statistical analyses included correlation, linear regression, and 3-way

analysis-of-variance (ANOVA; SAS Proc GLM) with fixed effects of taxon (T), collection site (S), year (Y), and their interactions on Pb and Ca concentrations and [Pb]/[Ca]. Results indicated that some analyses could be simplified by grouping sites as follows: Irondale (BR upstream of OLB); Desloge, FR, Cherokee Landing, and Mammoth Access (highly contaminated BR); House Springs (lower BR); and Rte. 66, Allenton, and Greentree (all MR). Results of these analyses also indicated that the Y main effect was not significant; site-year (S-Y) combinations were therefore considered rather than S and Y as separate factors. Neither Pb nor Ca was correlated with mean length or weight in either taxon, so length and weight were excluded from subsequent analyses.

The influence of Ca on spatial and temporal Pb patterns was assessed with analysis-of-covariance (ANCOVA; Hebert and Keenleyside 1995; Davis et al. 2013). The Ca concentrations were first centered (to geometric mean = 0.0) by subtracting the overall geometric mean (415 µg/g ww, n = 102) from the concentration in each sample. Simple linear regressions of log₁₀ Pb versus centered log₁₀ Ca for each taxon in each previously defined site group were then conducted, after which the Ca-normalized Pb concentration (PbCa) in each sample was estimated as the sum of its residual and the appropriate intercept. Log₁₀-transformed Pb, Ca, [Pb]/[Ca] and PbCa were analyzed with a general linear model (SAS Proc GLM) with S-Y, T, and S-Y*T interaction as fixed effects; and samples within S-Y and T as a random nested effect against which the fixed effects and interaction were evaluated. The *p* values for PbCa were corrected for the degrees-of-freedom (n = 8) used in the regressions. Differences among S-Y means were evaluated with Tukey's

test. Greentree Park data were included in the regression analyses but not in the ANCOVA because this site was sampled only in 2010, when only one sample of each species was collected. Unless otherwise indicated, a p value of $\alpha = 0.05$ was used to judge statistical significance.

Results and Discussion

Concentrations of Pb were typically <30 ng/g ww in all samples of both taxa from the MR and from the BR at Irondale (Fig. 2). Conversely, concentrations were >300 ng/g ww, the level that may trigger consumption advisories, in 21 of 42 samples (50 %; 14 of 21 redhorse, 7 of 21 sunfish) from the most heavily contaminated reach (Desloge to Mammoth Access) of the BR (Fig. 2). Concentrations at House Springs were generally intermediate; only one sample (sunfish) exceeded 300 ng/g ww (Fig. 2). At contaminated BR sites within and downstream of the OLB, where Pb concentrations were highest, fillet Ca explained 75 % to >99 % of the total variation in fillet Pb. Calcium explained only 18 %–61 % of the variation in samples from the MR and 3 %–9 % in samples from

Irondale, where Pb concentrations were lower (Fig. 2; Table 2).

The results for sunfish from Mammoth Access highlight the potential importance of Ca-normalizing fillet Pb concentrations. Mean Ca concentrations in Mammoth Access sunfish were 194 $\mu\text{g/g ww}$ in 2010 and 689 $\mu\text{g/g ww}$ in 2012. Mean Pb concentrations in these samples were 133 ng/g ww in 2010 and 382 ng/g ww in 2012, a difference of 2.9-fold. Mean PbCa was 253 ng/g ww in 2010 and 249 ng/g ww in 2012, which are nearly identical.

Although statistically significant (Table 3), fillet Ca differences between taxa were relatively small (geom. mean = 461 $\mu\text{g/g ww}$ for redhorse, 388 $\mu\text{g/g ww}$ for sunfish). Fillet Ca differences among S-Ys were also significant, as was S-Y*T interaction (Table 3). The interaction indicates that the Ca differences were not consistent among S-Ys and between taxa (Fig. 3a), which likely reflects differing preparation techniques. Fillet Pb differences between taxa and among S-Ys were also significant (Table 3). Fillet Pb concentrations were significantly greater at all S-Ys within and downstream of the OLB than in the MR or at Irondale (Fig. 3b). Differences among S-Ys for [Pb]/[Ca] were also significant, as were differences between taxa and the interaction, indicating that not all of the differences among S-Ys and between taxa were

Fig. 2 Fillet lead (Pb) and calcium (Ca) concentrations in redhorse and sunfish from four groups of sites on the Big, Flat, and Meramec rivers, 2005–2012. Numbered lines (1–8; lines 7 and 8 not visible) represent linear regressions developed for each group (see text and Table 2). Dashed reference line: Concentration (300 ng/g ww) that may trigger consumption advisories

Table 2 Results of simple linear regressions between $\log_{10}$ -transformed concentrations of lead and calcium in composite fillet samples of redhorse (RH) and longear sunfish (SF) from the indicated sites and groups of sites on the Big, Flat, and Meramec rivers

Group ^a	River(s)	Site(s)	Taxon	n	a	b	r ²	F	p
1	Big, Flat	Desloge–Mammoth	RH	21	2.445	1.113	0.80	74.3	<0.001
2	Big, Flat	Desloge–Mammoth	SF	23	2.358	0.842	0.75	64.1	<0.001
3	Big	House Springs	RH	8	1.984	1.317	>0.99	1545	<0.001
4	Big	House Springs	SF	6	1.969	0.980	0.84	21.0	0.010
5	Meramec	All	RH	13	1.267	1.702	0.61	17.4	0.002
6	Meramec	All	SF	15	1.117	0.026	0.02	2.95	0.110
7	Big	Irondale	RH	8	0.834	0.513	0.03	0.18	0.685
8	Big	Irondale	SF	8	0.927	0.743	0.09	0.56	0.481

n, Number of observations; a, intercept; b, regression coefficient (slope); r², coefficient of determination

^a Group numbers as indicated in Fig. 2

Table 3 Results of analysis-of-variance, as degrees-of-freedom (df), F and p values, and error mean-squares (ms) among site-years (S-Y), between fish taxa (T), and S-Y*T interaction for $\log_{10}$ transformed

calcium (Ca), lead (Pb), and Ca-normalized Pb (PbCa) concentrations and Pb:Ca molar ratios ([Pb]/[Ca]) in composite fillet samples

Source of variation	df	Ca		Pb		PbCa		[Pb]/[Ca]	
		F	p	F	p	F	p	F	p
S-Y	14	5.65	<0.001	33.6	<0.001	53.7	<0.001	56.0	<0.001
T	1	4.16	0.046	6.29	0.015	2.21	0.143	2.87	0.096
S-Y*T	14	3.31	<0.001	1.45	0.160	1.53	0.133	2.05	0.029
Sample ^{a, b}	60 ^b	3.56	0.017	3.14	0.027	2.80	0.040	2.68	0.046
Error ms ^c	10	0.035	–	0.290	–	0.049	–	0.047	–

^a Within S-Y and T

^b Sample df = 52 for PbCa

^c Among replicate analyses

accounted for by [Pb]/[Ca] (Table 3). Conversely, the S-Y main effect, but not T or S-Y*T, was significant for PbCa (Table 3), and PbCa in redhorse and sunfish from all BR sites within and downstream of the OLB were effectively identical from 2005 through 2012 (Fig. 3c). However, differences among replicate analyses were significant for Pb, Ca, [Pb]/[Ca], and PbCa (Table 3), indicating that not all of the among-sample differences in fillet Pb reflect Ca differences. These Ca differences are associated with unknown factors not related to sample preparation that were not included in the statistical model. Comparisons of PbCa S-Y means nevertheless reflect temporal and geographic trends without the confounding influence of taxon or sample preparation on Ca. In addition, the normalized concentrations are in units that can be used and interpreted by natural resource managers, environmental regulators, and the public. Although results for [Pb]/[Ca] were similar, the molar ratios are not directly useful. Moreover, because the relations between Pb and Ca are not isometric, normalization using ratios may yield counterintuitive results (Hebert and Keenleyside 1995).

As expected given that they are computed from the same values, PbCa and [Pb]/[Ca] were closely correlated. The

relation between PbCa and [Pb]/[Ca] was statistically significant ($p < 0.001$) and explained >98 % of the variation in PbCa (Fig. 4). Nearly all the predicted PbCa concentrations (and all predicted values >300 ng/g ww) were within the 95 % prediction region of the regression, and differences between redhorse and sunfish were minimal after Ca normalization (Fig. 4).

Fillet Pb concentrations in recently collected (2005–2012) redhorse and sunfish from the FR and the BR downstream of the OLB appear to be lower than they were historically. Arithmetic mean concentrations were typically 600–900 in suckers (three spp.) and 300–400 ng/g ww in sunfish during the early 1980s, and concentrations >300 ng/g ww were common in most of the BR downstream of the OLB (Schmitt et al. 1984; Czarnecki 1985; Schmitt and Finger 1987). By 1998–2000, arithmetic mean concentrations in the most contaminated reach of the BR and in the FR had declined to 190–470 ng/g ww in suckers and 100–240 ng/g ww in sunfish (Gale et al. 2004), which are similar to the geometric means for 2005–2012. However, the extent to which these differences reflect declining Pb concentrations is difficult to ascertain because preparation methods and sampling protocols differed among studies and Ca was not measured.

Fig. 3 Geometric mean (± 1 SE) fillet concentrations of **a** calcium (Ca), **b** lead (Pb), and **c** Ca-normalized Pb (PbCa) in redhorse and sunfish from site-years on the Big, Flat, and Meramec rivers, 2005–2012 (OLB Old Lead Belt). Site-years with the same letter(s) are not significantly different ($p < 0.05$). Dark vertical lines separate MR from BR sites. Dashed reference lines for Pb and PbCa: Concentration (300 ng/g ww) that may trigger consumption advisories

Schmitt and Finger (1987) measured fillet Pb and Ca concentrations and analyzed $[Pb]/[Ca]$ in redhorse collected from the BR during the early 1980s. Mean fillet $[Pb]/[Ca]$ was 344 in redhorse fillets from the BR at Desloge and 218–235 at two sites further downstream, which are greater than all S-Y means from the contaminated reach in 2005–2012 (max. = 264). Concentrations of Ca and $[Pb]/[Ca]$ were also determined in northern hog sucker (*Hypentelium nigricans*) fillets obtained from the BR near Cherokee Landing in 2005; Pb concentrations averaged 340 ng/g ww (estimated from dw concentrations and 80 % moisture) and $[Pb]/[Ca]$ averaged 132 (Schmitt et al. 2009), both of which are within the ranges of our 2005–2012 samples from the contaminated reach and lower than historical concentrations. Based on $[Pb]/[Ca]$ and its relation to PbCa (Fig. 4), we estimate that PbCa in redhorse from the most contaminated parts of the BR averaged 450–701 ng/g ww during the early 1980s (Schmitt and Finger 1987), declining to 300–500 ng/g ww in recently

collected (2005–2012) redhorse and sunfish. Estimated mean PbCa for the northern hog suckers analyzed by Schmitt et al. (2009) was 273 ng/g ww. In addition, fillet Pb was <300 ng/g ww in all redhorse from the lower BR (House Springs) in 2011 and 2012 (Fig. 2), and PbCa was <300 ng/g ww in all samples of both taxa (Fig. 3c). Concentrations of Pb normalized to the geometric mean Ca concentration tend to be lower than their respective original values because the geometric mean dampens the influence of extraordinarily large values, of which there were many due to the heterogeneous distribution of Ca-rich material in the samples. Depending on how the fish are prepared, PbCa computed from our equations may therefore underestimate the risk to human consumers. A more protective approach would be to normalize Pb to a higher Ca concentration (e.g., the 90th percentile Ca concentration), which could be easily accomplished from the information presented here.

Collectively, our results confirm previous findings that concentrations of Pb and Ca are correlated and that both are

Fig. 4 Calcium (Ca)-normalized lead (Pb) concentrations (PbCa) versus the molar ratio of Pb to Ca ($[Pb]/[Ca]$) in redhorse and sunfish fillets (regression $\pm$ 95 % prediction region). Dashed reference line: Concentration (300 ng/g ww) that may trigger consumption advisories

heterogeneously distributed. As reported for suckers (Schmitt and Finger 1987; Schmitt et al. 2007), the amount of Pb in fillet samples from Pb-contaminated sites depends on the amount of material other than muscle in the sample. We found this to be equally true for sunfish, which has not been previously reported. In addition, concentrations of Pb in redhorse and sunfish fillets were equivalent after accounting for differences in the Ca content of the samples. Nevertheless, and although Ca-normalized Pb concentrations are useful for monitoring, additional information is necessary if this method is used for human health risk assessment or establishing consumption advisories. Previous studies have shown that Pb concentrations in fish muscle, even from heavily contaminated sites, are low (Settle and Patterson 1980; Wiener 1982; Schmitt and Finger 1987; Schmitt et al. 2009). Concentrations in the parts of fish consumed by humans depend on how much material other than muscle is present, which in turn depends on how the fish are prepared. As such, measured Pb concentrations in our skinless, boneless fillet samples are undoubtedly lower than what is present in fish prepared in accordance with locally prevalent methods. In southern Missouri, catostomids and other “bony” species are often prepared as bone-in, skin-on fillets that are externally scored with a knife prior to deep-frying, a process that softens the bones and allows them to be eaten (Schmitt and Finger 1987). Concentrations of Ca and Pb in fillets prepared in this manner are likely to be higher than in skinless, boneless fillets. For example, the amount of Ca in northern

hog sucker fillets was profoundly influenced by where on the fish the fillet sample was obtained, no doubt reflecting the amount of bone it included (Schmitt et al. 2009). Elsewhere, eviscerated whole fish may be steamed, boiled, or smoked (e.g., Schmitt et al. 2006), in which case Ca and Pb concentrations may be higher. Regardless, whether the Pb is on or in the fillets or the fact that it may be associated with Ca is largely irrelevant to the fish consumer; it is all part of the portion and should be considered.

Reducing Pb concentrations in BR fish and rescinding the consumption advisory are goals of ongoing and future remediation in the OLB. The 2005–2012 data represent a useful benchmark against which to gauge improvement. Accurate assessment of progress based on the present monitoring design (3 composite samples of each taxon per site visit) will require control of variation and bias associated with Ca in the samples, which can be achieved via the Ca-normalization procedure described here. The procedure is reasonably straightforward, maximizes the information that can be extracted from the data, and is flexible; i.e., additional sites (as S-Ys) can be added without compromising the design and individual or multiple S-Ys can be compared as linear contrasts. In addition, statistical relations between Pb and Ca will become better defined as additional data are acquired under the consistent field protocol implemented in 2005. And finally, in-stream sediment is likely to be a focus of environmental remediation in the BR watershed. Future studies might therefore seek to establish relations between fillet PbCa and sediment Pb concentrations to focus and guide remediation.

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