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Selenium and Mercury in Freshwater Fish Muscle Tissue and Otoliths: A Comparative Analysis

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Abstract: Evaluating potential ecological and human health risks of exposure to bioaccumulative trace elements is typically implemented using analysis of tissue samples. Increasingly, the microchemistry of fish calcified structures is used to elucidate the lifetime exposure to trace elements. In the present study, we measured total mercury (THg), methylmercury (MeHg), and selenium (Se) in muscle tissue and otolith samples from 12 species of fish collected at reference sites and locations influenced by power plant wastewater. Muscle tissue concentrations of Se were sensitive to recent wastewater exposure magnitude, stream type, trophic level, and species ($p < 0.001$). For Hg, concentrations in muscle tissue and otoliths were affected only by trophic level and species. Levels of THg and Se in muscle tissue and otolith samples were positively correlated for those species with a robust sample size. Some individual fish from 3 species (channel catfish, hybrid striped bass, and freshwater drum) showed significantly increasing or decreasing lifetime concentrations of either THg or Se in otolith samples. Multiple regression analysis indicated that for bluegill muscle tissue Se concentrations could be best explained utilizing water concentrations of selenium, sulfate, and molybdenum ($r^2 = 0.87$; $p < 0.001$). Because of the increased cost and specialized sample processing requirements of analyzing trace elements in otolith structures, it may be prudent to limit these analyses to those species where insights into temporal trends are sought or where evidence indicates that fish move into or out of contaminated water bodies. *Environ Toxicol Chem* 2019;38:1467–1475. © 2019 SETAC

Keywords: Bioaccumulation; Otoliths; Mercury; Selenium

INTRODUCTION

Environmental monitoring programs typically use quantitative measurements of contaminants in various biotic and abiotic compartments to assess potential risk to ecological and human receptors. The measurement of contaminants in abiotic samples (e.g., water column and sediment) is advantageous as a result of the relative ease of sample collection and the lower cost per sample. Nonetheless, the analysis of biological tissues provides a more robust and direct estimate of exposure from both dietary and aqueous sources. The concept of the tissue residue approach and its advantageous technical attributes have been reviewed (Meador et al. 2008; Sappington et al. 2010). For the bioaccumulative trace elements mercury (Hg) and selenium (Se), numerous researchers have argued that tissue residue concentrations, as opposed to ambient aqueous concentrations, are better predictors of potential risk to fish and wildlife and their consumers (Wiener et al. 2007; Hodson et al. 2010).

Microchemistry analyses of fish hard parts (otoliths, statoliths, scales, and fin rays) offer a useful tool for inferring the history of environmental exposures of marine and freshwater fish (Campana and Thorrold 2001; Pracheil et al. 2014). Whereas otolith analysis is used frequently to elucidate various life history attributes (often for specific fish species stocks), to an increasing extent the elemental composition of these structures is used to determine temporal and spatial patterns of contaminant exposure (e.g., Friedrich and Halden 2010; Søndergaard et al. 2015; Selleslagh et al. 2016). Otolith microchemistry may allow a reliable lifetime chronology of trace element exposure. However, key assumptions are that trace elements are deposited in calcium carbonate layers in proportion to concentrations in the ambient water and that the elements in the matrix are stable once deposited (Elsdon et al.

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2008). Steady-state exposure conditions are relatively rare, however, and thus habitats with distinct chemical profiles (e.g., reference vs contaminated) are needed to presume potential fish movement between these.

The analysis of trace element profiles in fish muscle tissues and otolith structures has contrasting exposure scales. In general, the measurement of Hg and Se in soft muscle tissue represents exposure intervals of less than 1 yr, whereas profiles in otoliths account for lifetime exposure. The objective of the present study was to evaluate the utility of measuring trace elements in both muscle tissue and otolith samples in individual fish to provide greater insights into exposure and bioaccumulation patterns. Fish (encompassing a total of 12 species) were collected from Ohio River basin (USA) reference sites and sites influenced by coal-fired, power plant wastewater. For each fish collected, we measured Se and Hg concentrations in muscle tissue and otolith samples. We sought to compare exposure profiles for the 2 tissue types and identify ecological and water quality factors affecting bioaccumulation patterns.

MATERIALS AND METHODS

Study location

The present study location was the Ohio River and its tributary streams. Fish and water quality samples were collected from 7 sites: 2 reference areas (one in the Ohio River and one in the mouth of a tributary) and 5 exposure locations. Exposure sites (2 in West Virginia, 2 in Kentucky, and one in Ohio) were those influenced by coal-fired, power plant wastewater (flue gas desulfurization [FGD] and/or fly ash wastewater). These sites varied in receiving stream instream waste concentration (IWC) percentages. Figure 1 indicates locations of the sampling areas and the power plants.

All of the power plants were equipped with state-of-the-art air pollution control technologies: cold-side electrostatic precipitators (fly ash removal), selective catalytic reduction systems (flue gas NO_X capture), and wet FGD systems for enhanced SO_2 removal. Table 1 details the name of each facility, generating capacity, wastewater type, wastewater receiving stream, and exposure type. The exposure type was based on the level of wastewater discharge influence at the point of sampling. For example, a high-exposure location was an effluent-dominated area (IWC ≥ 50%). Flue gas desulfurization wastewater and FGD landfill leachate were characterized by high total dissolved solids with moderate-to-high enrichment of selected trace elements including arsenic, Hg, and Se (Electric Power Research Institute [EPRI] 2006; Kosson et al. 2009; Ohio River Valley Water Sanitation Commission [ORSANCO] 2013).

Water sampling and analysis

Grab water samples (typically at mid-depth) were collected at each study site just before fish collection. Trace elements (US

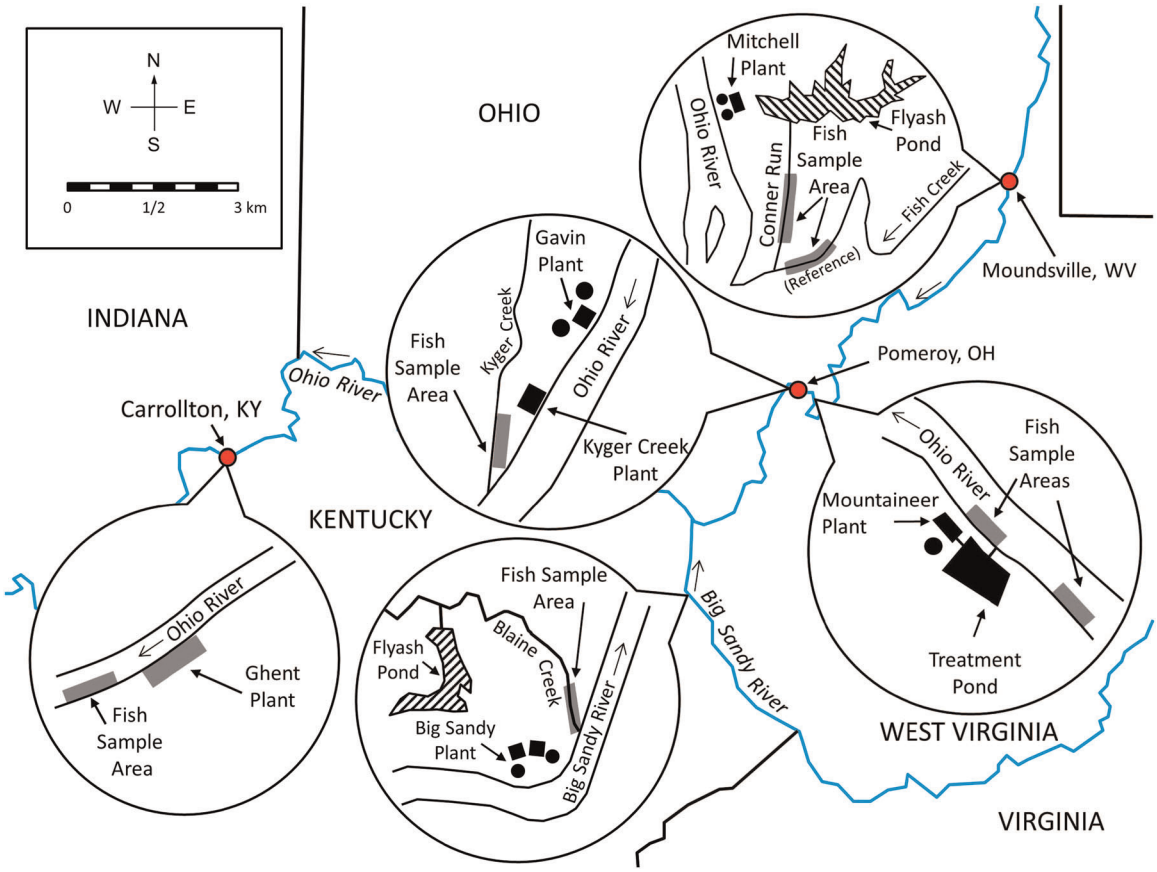

FIGURE 1: Location of fish and water sampling locations in the Ohio River and tributary streams.

TABLE 1: Reference and power plant wastewater exposure fish and water sampling locations on the Ohio River and tributary streams

Sample location	Exposure type	Generating facility	Facility generating capacity (MW)	Wastewater	IWC[a] (%)
Fish Creek, WV	Reference	NA	NA	NA	0
Ohio River—river mile 239, WV	Reference	NA	NA	NA	0
Ohio River—river mile 536, KY	Low	Ghent	1932	Treated FGD wastewater	5
Ohio River—river mile 242, WV	Medium	Mountaineer	1300	Treated FGD/fly ash leachate wastewater	33
Blaine Creek, KY	Medium	Big Sandy	1060	Treated fly ash wastewater	30
Kyger Creek, OH	High	Gavin/Kyger Creek plants	2600/1085	Treated FGD leachate/fly ash wastewater	45
Connor Run, WV	High	Mitchell	1600	Treated fly ash	95

[a]Percentage of wastewater discharge flow to receiving stream flow.
MW = megawatts; IWC = instream waste concentration; NA = not applicable; FGD = flue gas desulfurization; WV = West Virginia; KY = Kentucky; OH = Ohio.

Environmental Protection Agency [USEPA] Method 200.8), ions (USEPA Method 200.7), and various routine water quality variables were quantified. Samples for the analysis of low-level Hg were not collected; and no water quality data were available for the one low-exposure sampling location. Historical wastewater discharge water quality data were obtained for effluent-dominated sites. These sites represented National Pollutant Discharge Elimination System monitoring locations. For fish sampling locations located on the Ohio River, water quality data were obtained from the Ohio River Valley Water Sanitation Commission (2013) fixed water quality stations at lock and dam sites located just upstream of the fish sampling sites.

Fish collection

Fish were collected using DC-pulsed electrofishing during October 2013. Before fish sampling, pH (S.U.), temperature (°C), and specific conductivity (μhmos/cm) were measured. The target species were those considered trophic level 3 (omnivores) and trophic level 4 (carnivores). Each fish retained was measured for total length (mm) and wet weight (g). Fish heads were excised and placed in labeled plastic bags and frozen in the field. Muscle tissue samples (skin-off, right and left dorsal samples combined) were removed from each fish. These samples were rinsed with distilled water, wrapped in hexane-rinsed aluminum foil and then in plastic bags, and placed in dry ice. Scales or pectoral spine sections were obtained for age determination of each fish retained. A total of 93 fish representing 12 species were analyzed for trace element concentrations in muscle tissue and otolith samples.

Laboratory analysis

For each fish sample, sagittal otoliths were extracted from the heads, dried, and placed in labeled envelopes. The sagittal otoliths were embedded in epoxy resin and cut to create a dorso-ventral cross section through the core of the otolith, exposing all annuli. The posterior half was embedded in 25-mm Lucite microprobe mounts and hand polished. Quantification of total mercury (THg) and Se in the otoliths was conducted using laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) using a Merchantek LUV 213 Nd:YAG nanosecond laser coupled to a Thermo Scientific Finnigan Element 2 ICP-MS. An 80-μm diameter beam traveling 4 μm/s scanned the otolith from core to edge using a high angle to the annuli. Calcium, as 56 wt % CaO, was used as an internal standard, and MACS-3 pressed pellet synthetic carbonate was used as an external standard. Concentrations of trace elements and detection limits were processed with Iolite Ver 2.21 Ref software (Paton et al. 2011) and exported to Microsoft Excel for final presentation. Results of analyses of THg and Se in otolith samples were expressed as dry weight.

Muscle tissue samples for each fish were shipped to Brooks Applied Laboratories. The samples were homogenized and analyzed for THg, methylmercury (MeHg), and Se. Total mercury, MeHg, and Se were analyzed using USEPA Methods 1631 and 1630, as well as 1638 dynamic reaction cell technology, respectively. The solids content of the samples was analyzed by National Environmental Monitoring Index Standard Method 2540G, with samples dried at approximately 100 °C for 4 d. Samples analyzed by USEPA Method 1631 were acid digested and further oxidized with BrCl. The samples were analyzed by $SnCl_2$ reduction, followed by gold amalgamation, thermal desorption, and cold vapor atomic fluorescence spectroscopy (CVAFS) utilizing a Brooks Rand Instruments MERX-T analyzer. Samples examined by USEPA Method 1630 were digested in a KOH/methanol solution. These samples were then evaluated by ethylation, Tenax trap pre-concentration, gas chromatography separation, pyrolytic combustion, and analyzed by CVAFS using a Brooks Rand Instruments MERX-M analyzer. Samples analyzed by USEPA Method 1638 dynamic reaction cell technology were hot-block digested with HNO_3 and hydrogen peroxide (H_2O_2), then diluted to volume with deionized water. The digests were then analyzed by ICP-MS employing dynamic reaction cell technology to reduce potential polyatomic interferences and to achieve lower method detection limit values.

Assurance and quality control analyses conducted on each shipped batch included analyses of method blanks, certified reference materials (DORM-3 and IAEA 407), duplicates, matrix spikes, and matrix spike duplicates. A detectable value was defined as a value measured at or above the method detection limit. Results of analyses for THg and MeHg in muscle tissue

samples were expressed as wet weight, whereas outcomes for Se were expressed as dry weight.

Data analysis

Results were analyzed to evaluate: 1) the similarity/correlation, if any, of Hg and Se concentrations in muscle tissue and otolith samples, 2) environmental and ecological factors potentially associated with amounts of Hg and Se in the 2 sample types (i.e., exposure regime, species tested, trophic level, site location), 3) temporal patterns of Hg and Se accumulations in otolith samples during the entire exposure history of selected fish, and 4) the exceedance of muscle tissue toxicity thresholds for Hg (human health protection) and Se (aquatic life protection). Multivariate analysis of variance (MANOVA) was used to assess factors associated with concentrations of THg and Se in both muscle tissue and otolith samples. The MANOVA assumptions of normality and homogeneity of variance in each cell were tested by examining the MANOVA residuals (Ott and Longnecker 2010). Box plots, normal probability plot, and the MANOVA diagnostics from R were used to verify the normality and homogeneity of variance assumptions (Ott and Longnecker 2010; R Development Core Team 2018). The MANOVA residuals were found to be consistent with the assumptions. Average otolith trace element concentration data for the most recent year (2013) were used to represent contemporary exposure conditions. For otolith sample results with multiple years of exposure for a given fish, temporal trends in trace element concentrations were tested using Kendall's tau nonparametric trend test.

Multivariate linear models were used to explore the hypothesis that concentrations of THg and Se in muscle tissue and otolith samples could be explained by ecological and water quality variables. Several explanatory variables were tested employing exploratory regression analysis. Variables were added iteratively and removed from the regression model; changes in R^2 values and the Akaike information criterion (AIC; Venables and Ripley 2013) were subsequently monitored. The AIC describes the fit of various models while also considering model complexity, and was derived using the equation AIC = 2K – 2ln(L), where K is the number of parameters and L is the maximum likelihood.

RESULTS AND DISCUSSION

Water analyses

Table 2 indicates mean concentrations of 4 trace elements (total recoverable analyses) and 3 routine variables in water samples collected from each exposure category stream ($N = 1$–3 samples per location). The 2 high exposure sites had relatively elevated concentrations of arsenic, molybdenum, Se, and total dissolved solids. Levels of most other trace elements were generally low (<5 μg^{-1}) or less than detection at all locations.

Tissue and otolith trace element concentrations

All of the quality assurance and control analyses were within the specific laboratory criteria: duplicates (68 analyses; criteria recovery limit 30–35% difference), certified reference materials (14 analyses; criteria recovery limit 65–135%), matrix spikes (29 analyses; criteria recovery limit 65–135%), matrix spike duplicates (30 analyses; criteria recovery limit 65–135%), and method blanks (37 analyses; maximum detection limit varied with each trace element). Method detection limits ranged from 0.10 to 3.94 ng/g (THg); 0.63 to 1.59 ng/g (MeHg); and 0.05 to 0.07 mg/kg (Se). Geometric mean muscle tissue concentrations of THg, MeHg, and Se for the 8 most commonly collected species are provided in Table 3. Other fish species were collected for tissue analysis; however, there were few samples collected from these ($N < 3$); thus they were excluded. Three fish species (white sucker, green sunfish, and freshwater drum) for individuals gathered at high exposure locations had geometric mean muscle tissue Se concentrations that exceeded USEPA's muscle/fillet Se criterion of 11.3 mg/kg dry weight (US Environmental Protection Agency 2016). Bluegills collected from high exposure sites had a geometric mean muscle tissue Se concentration slightly less than the USEPA criterion (10.3 mg/kg). The mean muscle tissue Se concentration of white suckers in the present study was consistent with elevated accumulations of Se documented for this species gathered from locations in Colorado having very high ambient water concentrations of Se (Canton 2010). Hybrid striped bass collected from high exposure sites had a geometric mean MeHg accumulation that exceeded USEPA's MeHg human health water quality criterion of 0.3 mg/kg wet weight (US Environmental Protection Agency 2001). One factor that could have affected measured concentrations of THg and MeHg in muscle tissue samples was potential in vivo interactions between Se and Hg. Selenium has been shown to antagonize Hg accumulation in aquatic organisms (e.g., Belzile et al. 2006).

The muscle tissue trace element concentrations reported in the present study were generally similar to previous studies documenting Se and Hg fish tissue amounts for fish collected at reference locations in the Ohio River (Walters et al. 2010; Reash et al. 2015). In contrast, concentrations of THg in Ohio River

TABLE 2: Mean concentrations of selected water quality variables at differing exposure locations[a]

Exposure	As (μg^{-1})	Cu (μg^{-1})	Mo (μg^{-1})	Se (μg^{-1})	Alkalinity (mg^{-1})	Total hardness (mg^{-1} $CaCO_3$)	Total dissolved solids (mg^{-1})
Reference	1.0 (0.1[a])	2.2 (0.6)	2.8 (NA[b])	0.5 (0.2)	122 (NA)	112 (21)	184 (NA)
Medium	5.2 (2.9)	11.1 (10.8)	20.8 (NA)	3.6 (0.7)	55 (NA)	81.3 (NA)	152 (NA)
High	22.6 (46.7)	1.9 (0.2)	155.7 (59.8)	34.9 (31.5)	50 (46.7)	246 (NA)	662.8 (NA)

[a]Standard deviations appear in parentheses.
[b]When mean value was based on one sample, NA (not applicable) applies.
As = arsenic; Cu = copper; Mo = molybdenum; Se = selenium.

TABLE 3: Geometric mean[a] muscle concentrations of selenium, total mercury, and methylmercury in fish collected from reference and wastewater exposure streams

Species	Exposure	*n*	Se[b] (mg/kg)	THg[c] (ng/g)	MeHg[c] (ng/g)
White sucker	High	5	38.93 (±4.72)	0.43 (±0.13)	ND
Channel catfish	Reference	8	1.43 (±0.34)	123.4 (±120.5)	111.1 (±105.8)
	High	5	3.65 (±3.84)	86.0 (±34.0)	78.3 (±27.9)
Hybrid striped bass	Reference	9	3.83 (±0.75)	134.2 (±45.8)	121.4 (±48.5)
	Medium	5	5.13 (±2.16)	188.2 (±96.7)	157.4 (±85.7)
	High	6	4.26 (±0.79)	409.0 (±88.7)	395.3 (±94.6)
Bluegill	Reference	10	4.92 (±6.05)	41.6 (±24.1)	36.8 (±39.4)
	Medium	10	3.50 (±2.32)	33.2 (±23.2)	30.4 (±20.7)
	High	8	10.28 (±15.78)	5.3 (±5.5)	4.2 (±5.0)
Longear sunfish	Reference	5	3.31 (±0.31)	73.1 (±16.0)	59.6 (±67.7)
	Medium	5	3.99 (±0.51)	47.7 (±15.7)	40.4 (±15.9)
Green sunfish	High	6	28.38 (±3.65)	3.13 (±2.9)	2.4 (±3.1)
Spotted bass	Reference	3	4.25 (±2.00)	139.0 (±(90.8)	99.3 (±59.7)
	Medium	5	4.64 (±0.87)	104.4 (±34.2)	113.3 (±29.0)
Freshwater drum	Medium	3	3.53 (±2.56)	102.2 (±259.6)	58.9 (±226.8)
	High	3	16.24 (±7.78)	111.7 (±46.2)	97.8 (±38.4)

[a]± Standard deviation.
[b]Concentration as dry weight.
[c]Concentration as wet weight.
Se = selenium; THg = total mercury; MeHg = methylmercury; ND = not detected.

hybrid striped bass recorded by Emery and Spaeth (2011) were considerably higher relative to accumulations in the present study.

Multivariate analysis of variance permutations were conducted to evaluate which ecological factors affected muscle tissue concentrations of Se and THg. The ecological variables used in the analysis were fish species, trophic level, exposure group, and location (actual site sampled). The distribution of residuals was examined to study the MANOVA assumptions and the residuals were found to have approximate normal distribution. Table 4 presents these results. For both Se and THg, trophic position and taxonomic identity were significant in the MANOVA. Whereas variations in THg muscle tissue concentrations were expected to change with trophic position because of well-documented biomagnification effects, the same was not observed with Se (Presser and Luoma 2010; DeForest et al. 2015). Fish length was significant for THg but not for Se. Both exposure group and location were significant in the MANOVA on muscle tissue Se concentrations but not for THg levels. This suggests that the dynamics of bioaccumulation of Se and THg in fish have dissimilarities (e.g., Se is a required essential trace nutrient and biomagnification trophic multiplier factors are relatively low).

Figure 2 represents the distribution of otolith THg and Se accumulations (all species combined) segregated by exposure type. As expected, the highest median values of THg and Se in otolith samples were in fish from high exposure sites, although the separation among exposure types was more pronounced in concentrations of Se. The species having the highest average THg amounts in the 3 exposure types were: reference—freshwater drum, 0.08 μg/g; medium—spotted bass, 0.20 μg/g; and high—hybrid striped bass/smallmouth bass, 0.08 μg/g. For otolith Se concentrations, the species with the highest average concentrations were: reference—small channel catfish, 1.4 μg/g; medium—large channel catfish, 1.5 μg/g; and high—white sucker, 13.4 μg/g. Table 5 displays results of the MANOVA analyses (all species combined). Significant variables in the MANOVAs for THg and Se in otolith samples generally paralleled those factors affecting levels in muscle tissue samples. For THg in otoliths, fish species was the only variable having a significant effect. For Se, species and trophic level both had a significant effect on otolith concentrations. These results suggest that, at least for the present study, variables influencing the bioaccumulation of either THg or Se in both soft tissue (muscle) and calcified structures (otoliths) somewhat mirror each other.

Correlations among tissue types

Parametric or nonparametric correlation analyses (based on tests of normality) were conducted to evaluate the relationship between muscle tissue and otolith trace element concentrations. For all species combined, collections of THg in muscle tissue and otolith samples (last 12 mo of exposure) were not

TABLE 4: Results of analysis of variances on factors influencing fish muscle tissue concentrations of selenium and total mercury

	Se in muscle tissue			THg in muscle tissue		
Ecological variable	MSE	*F* value	*p* value	MSE	*F* value	*p* value
Species	705	6.07	<0.001	24 547	3.34	<0.001
Trophic level	1581	136	<0.001	524 810	71.4	<0.001
Fish length	3	3.4	NS*	451 249	148	<0.001
Exposure group	368	31.7	<0.001	10 506	1.43	NS*
Location	479	41.2	<0.001	9764	1.33	NS*

*Not significant (NS) at $p > 0.05$.
Se = selenium; THg = total mercury; MSE = mean squared error.

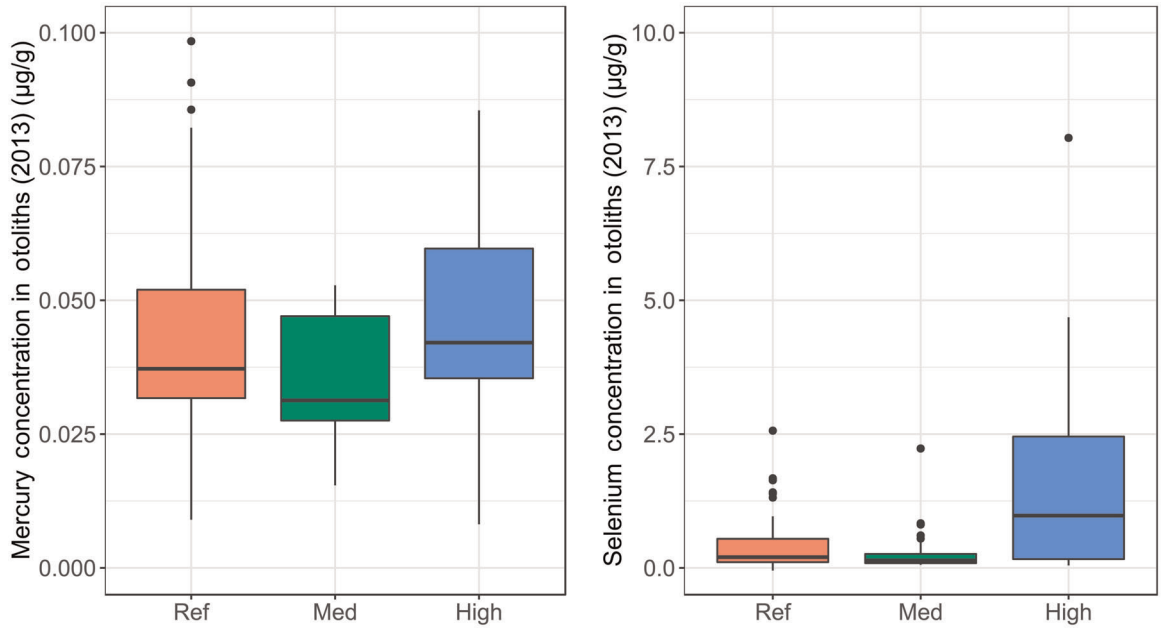

FIGURE 2: Box plots of the concentrations of mercury and selenium in otolith samples (all species combined) segregated by power plant wastewater exposure type. Within each box the horizontal line represents median values.

significantly correlated (rho = 0.04; $p > 0.05$), as were Se accumulation levels in muscle tissue and otolith samples (rho = –0.02; $p > 0.05$). In contrast, for 2 species that had a relatively large sample size (see Table 3), there was a significant correlation of THg concentrations between muscle tissue and otolith samples (hybrid striped bass; $p < 0.001$) and a significant correlation in Se levels between the 2 sample types for bluegill ($p < 0.05$).

TABLE 5: Results of analysis of variances on factors influencing fish otolith concentrations of selenium and total mercury[a]

	Se in otolith			THg in otolith		
Ecological variable	MSE	*F* value	*p* value	MSE	*F* value	*p* value
Species	705	60.7	<0.001	0.006	3.18	<0.01
Trophic level	1581	136	<0.001	0.002	0.89	NS*
Exposure group	368	31.7	<0.001	0.004	1.95	NS*
Location	479	41.2	<0.001	0.005	2.46	NS*

[a]All species combined.
*Not significant (NS) at $p > 0.05$.
Se = selenium; THg = total mercury; MSE = mean squared error.

Time series analysis

In fish for which otolith trace element concentration data were available for at least 5 yr (i.e., 2009–2013), a time series analysis was conducted to assess temporal trends. Of the 17 fish evaluated for temporal trends, 9 of these indicated no trends for both THg and Se concentrations. Eight fish, representing 3 species, showed significant increasing or decreasing levels of either THg or Se (Table 6). Four of these fish had increasing concentrations of THg, whereas 3 fish had significantly decreasing levels. Figure 3 illustrates a time series of measured THg levels in a freshwater drum otolith with significantly decreasing concentrations (tau = –0.75; $p < 0.001$). For Se, 5 fish (4 of these freshwater drum) had significantly

TABLE 6: Individual fish indicating significant temporal trends of total mercury or selenium concentrations as measured in otoliths

			Se in otolith		THg in otolith	
Species	Exposure type	Fish age (yr)	Kendall tau	*p* value	Kendall tau	*p* value
Channel catfish	Reference	8	—	—	–0.86	<0.01
Channel catfish	Reference	8	—	—	–0.71	<0.05
Channel catfish	High	7	0.81	<0.05	—	—
Hybrid striped bass	High	5	—	—	1.00	<0.05
Freshwater drum	Reference	17	0.63	<0.001	0.60	<0.001
Freshwater drum	High	7	0.90	<0.01	0.81	<0.05
Freshwater drum	High	9	1.00	<0.001	0.61	<0.05
Freshwater drum	Medium	18	0.58	<0.001	–0.75	<0.001

Se = selenium; THg = total mercury; Kendall tau = Kendall's tau nonparametric trend test.

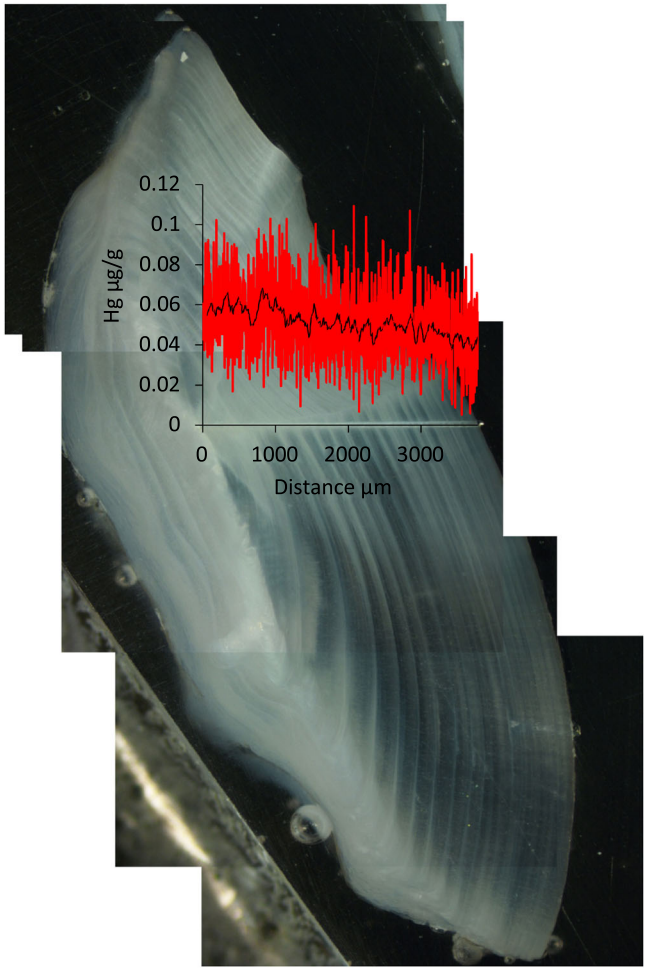

FIGURE 3: Laser ablation profile of lifetime concentrations of mercury (Hg) in otolith from a freshwater drum (age = 18+ yr) collected at an Ohio River medium exposure location.

increasing concentrations. Figure 4 illustrates a time series of measured Se levels in a freshwater drum otolith with significantly increasing Se concentrations (tau = 1.00; $p < 0.001$). These results suggest that the detection of significant temporal trends in contaminant levels for a particular fish species in a given water body should assume divergent temporal patterns among individual fish. Moreover, the practice of analyzing composite soft tissue samples (a convenient approach from a cost perspective) essentially masks potential trends exhibited by individual fish. The chemical analysis of calcified structures appears to have advantages in detecting real temporal trends relative to the analysis of pollutants in the soft tissues of individual fish (or composites) over a multi-year time series. The actual objectives of a study (and associated cost limitations), however, are important factors in deciding which tissue type is analyzed.

A potentially confounding factor in evaluating time series profiles is the possibility of fish movement between reference and wastewater-influenced reaches, or vice versa. We observed strikingly divergent temporal otolith trace element concentration profiles in 2 of 93 fish, suggesting that the movement of fish between differing water quality locations was relatively rare. This low frequency of divergent temporal profiles of Se or THg concentrations in otolith samples suggest that the majority of fish collected for analysis were residents of the locations in which they were found. Five of the 8 species from which muscle tissue trace element concentrations were provided (Table 3) were sunfish species. Sunfish in general tend to have limited home ranges (Fish and Savitz 1983).

Multivariate modeling of explanatory variables

Multivariate linear models were evaluated for species from which a relatively large sample size of muscle tissue results was available. Tissue trace element results were matched with water quality variable data to determine if a suite of these parameters could explain variation in tissue concentrations. For muscle THg results, no significant ($p < 0.05$) regression equations were identified considering all species analyzed.

For Se in muscle tissue samples, the best fit explanatory equation was for bluegill. A regression model including the water quality variables of total Se, sulfate, and molybdenum explained 87% of the variability in bluegill tissue concentrations ($F = 34.2$; *df*

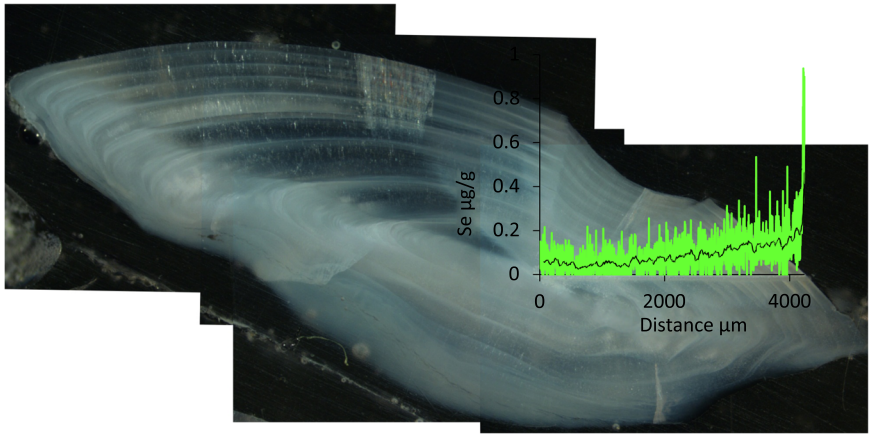

FIGURE 4: Laser ablation profile of lifetime concentrations of selenium (Se) in otolith from a freshwater drum (age = 9+ yr) collected at an Ohio River tributary stream high exposure site.

[3, 15]; $p < 0.001$). These same water quality variables were components of a multiple regression model that explained 96% of variation in whole-body Se concentrations for 3 species of *Lepomis* spp. sunfish, including bluegill (Reash 2012). Three of the study locations used in the present study (Blaine Creek, Connor Run, and Fish Creek) were also sampled in Reash (2012).

Previous similar studies

Previous reports on concomitant analyses of the 2 tissue types in individual fish are scarce. Selleslagh et al. (2016) reported levels of various trace elements in juvenile flounder otoliths and different soft tissues. In general, the levels in the 2 tissue types reflected the laboratory exposure regimes.

CONCLUSIONS

The present study indicated useful comparisons of delineating patterns of bioaccumulation for bioaccumulative trace elements using the analysis of both soft tissue and hard tissue. In the present study, concentrations of Se and THg in field-collected fish were correlated between otolith and muscle tissue samples—but only for species with a robust sample size. The analyses of otolith Se and THg levels, at least for fish that were relatively old, proved successful in discerning long-term temporal trends of exposure. Not unexpectedly, fish collected from high exposure sites had significantly elevated Se and/or THg concentrations.

The ecological and exposure factors that were associated with concentrations in muscle tissues and otoliths differed between Se and THg. Concentrations of Se in muscle tissues were affected by species, trophic level, exposure type, and site location; however, in otoliths only species and trophic level were significant. Total Hg levels in otoliths were affected only by species. These differences may be explained by the manner in which the 2 trace elements bioaccumulate. For Hg, it may be that dissimilarities in feeding ecology among the various species could explain the relative unimportance of water quality (exposure) and other site-specific factors.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4432.

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Data Accessibility—Data obtained during the present study are accessible from the corresponding author (rjreash@aep.com).

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