2007

Concentrations of Metals in Aquatic Invertebrates from the Ozark National Scenic Riverways, Missouri

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Prepared in cooperation with the National Park Service

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Open-File Report 2007–1435

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Cover Photographs. Shells of Asian clams (*Corbicula fluminea*) on the bottom of a stream (background). Photograph by B. Poulton, U.S. Geological Survey. Asian clams, *Corbicula fluminea* (top left inset). Photograph by B. Poulton, U.S. Geological Survey. The Current River at Cave Spring (right inset). Photograph by D. Hardesty, U.S. Geological Survey. Golden crayfish, *Orconectes luteus* (bottom left inset). Photograph courtesy of R. DiStefano, Missouri Department of Conservation.

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U.S. Department of the Interior
DIRK KEMPTHORNE, Secretary

U.S. Geological Survey
Mark D. Myers, Director

U.S. Geological Survey, Reston, Virginia: 2007

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Suggested citation:
Schmitt, C.J., Brumbaugh, W.G, Besser, J.M., and May, T.W., 2007, Concentrations of metals in aquatic Invertebrates from the Ozark National Scenic Riverways, Missouri: U.S. Geological Survey Open-File Report 2007–1435, 23 p. with appendixes.

Acknowledgements

This study was supported by the Contaminants Biology and Inventory and Monitoring Programs of the U.S. Geological Survey (USGS) and the National Park Service (NPS) through an interagency agreement. Field collections were conducted under the authority of a Scientific Investigator's Permit from the NPS. V. Grant, M. Gossett, J. Luraas, and E. Kunz (NPS); M. Mac, B. Poulton, and S. Olson (USGS); J. Ackerson, D. Mayer, R. Legler, and M. Reed (Missouri Department of Conservation, MDC); and R. Sarver and K. Lister (Missouri Department of Natural Resources) assisted in the field. J. Arms, S. Koppi, and V. Melton (USGS) assisted in the laboratory. M. Struckhoff (USGS) prepared the map. J. Petersen (USGS) provided the NAWQA data. A.L. Allert (USGS), J. K. Crawford (USGS), D. Bowles (NPS), J. Petersen, and M. McKee (MDC) provided useful comments on earlier versions of this report.

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Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
millimeter (mm)	0.03937	inch (in.)
micrometer	0.0000393	inch (in)
meter (m)	3.218	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
milliliter (mL)	.034	ounce, fluid (fl. oz)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
milligram (mg)	0.000035	ounce (oz)
	Concentration	
microgram per gram (µg/g)	=	part per million (ppm, 106)
	Area	
hectare (ha)	2.47	acres (a)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8×°C)+32$$

Concentrations of metals are given in micrograms per gram (µg/g).

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Abstract

This report summarizes the findings of a study conducted as a pilot for part of a park-wide monitoring program being developed for the Ozark National Scenic Riverways (ONSR) of southeastern Missouri. The objective was to evaluate using crayfish (*Orconectes* spp.) and Asian clam (*Corbicula fluminea*) for monitoring concentrations of metals associated with lead-zinc mining. Lead-zinc mining presently (2007) occurs near the ONSR and additional mining has been proposed. Three composite samples of each type (crayfish and Asian clam), each comprising ten animals of approximately the same size, were collected during late summer and early fall of 2005 from five sites on the Current River and Jacks Fork within the ONSR and from one site on the Eleven Point River and the Big River, which are outside the ONSR. The Big River has been contaminated by mine tailings from historical lead-zinc mining. Samples were analyzed by inductively coupled plasma mass spectrometry for lead, zinc, cadmium, cobalt, and nickel concentrations. All five metals were detected in all samples; concentrations were greatest in samples of both types from the Big River, and lowest in samples from sites within the ONSR. Concentrations of zinc and cadmium typically were greater in Asian clams than in crayfish, but differences were less evident for the other metals. In addition, differences among sites were small for cobalt in Asian clams and for zinc in crayfish, indicating that these metals are internally regulated to some extent. Consequently, both sample types are recommended for monitoring. Concentrations of metals in crayfish and Asian clams were consistent with those reported by other studies and programs that sampled streams in southeast Missouri.

Introduction

The Ozark National Scenic Riverways (ONSR) lies within the Ozark Plateau in southeastern Missouri (fig. 1). The ONSR, which is managed by the National Park Service (NPS), comprises approximately 33,265 ha and includes 216 km of the Current River and Jacks Fork. The ONSR is famous for its large freshwater springs, caves, spring-fed rivers, and recreational opportunities in a landscape of oak-hickory forest and pastoral river valleys. Canoeing, camping, and sport fishing are especially popular recreational activities. The Current River and Jacks Fork support populations of Ozark hellbender (*Cryptobranchus bishopi*), a large, predatory salamander considered endangered in Missouri and that has been proposed for Federal listing (Missouri Department of Conservation, 2006).

The highly mineralized Ozark Plateau contains economically significant lead-zinc deposits of the Mississippi Valley Type (Goldhaber and others, 1995). Surface lead-zinc deposits were discovered by early French explorers; these and subsequently discovered deposits have been exploited at varying levels of intensity since the early 1700s. Advances in mining technology facilitated deep mining, which was and remains focused in two southeast Missouri districts: the “Old Lead Belt”, primarily located in Washington and St. Francois counties, which was active from about 1700 until the early 1970s (Schmitt and others, 1984; Gale and others, 2004); and the “New Lead Belt”, primarily located in Crawford, Iron, and Reynolds counties, which became active in the 1960s and where lead, zinc, copper, and other metals are still mined (Wixson and Jennett, 1975; Wixson, 1978; Proctor, 1984; Imes, 2002). These metals, along with other potentially toxic byproduct metals including cadmium, cobalt, and nickel, are released to the environment from mining and ore processing. Most mining and ore processing in the New Lead Belt occurs on lands that formerly were part of the Mark Twain National Forest. The southernmost extent of the New Lead Belt is located only about 32 km northeast of the ONSR. Additional potentially exploitable deposits have been discovered within the Mark Twain National Forest in Shannon County (Imes, 2002). Although the prospecting area lies in the surface water drainage of the Eleven Point River, it is in the ground water recharge area of Big Spring, an important feature within ONSR that contributes substantially to flows in the Current River (Imes, 2002; Imes and others, 2007).

Ambient concentrations of lead and other metals from natural sources in the Ozarks have been augmented by releases from mining and ore processing activities. Karst features such as sinkholes, conduits, and springs in the area may facilitate the transport of mining-associated contaminants from runoff

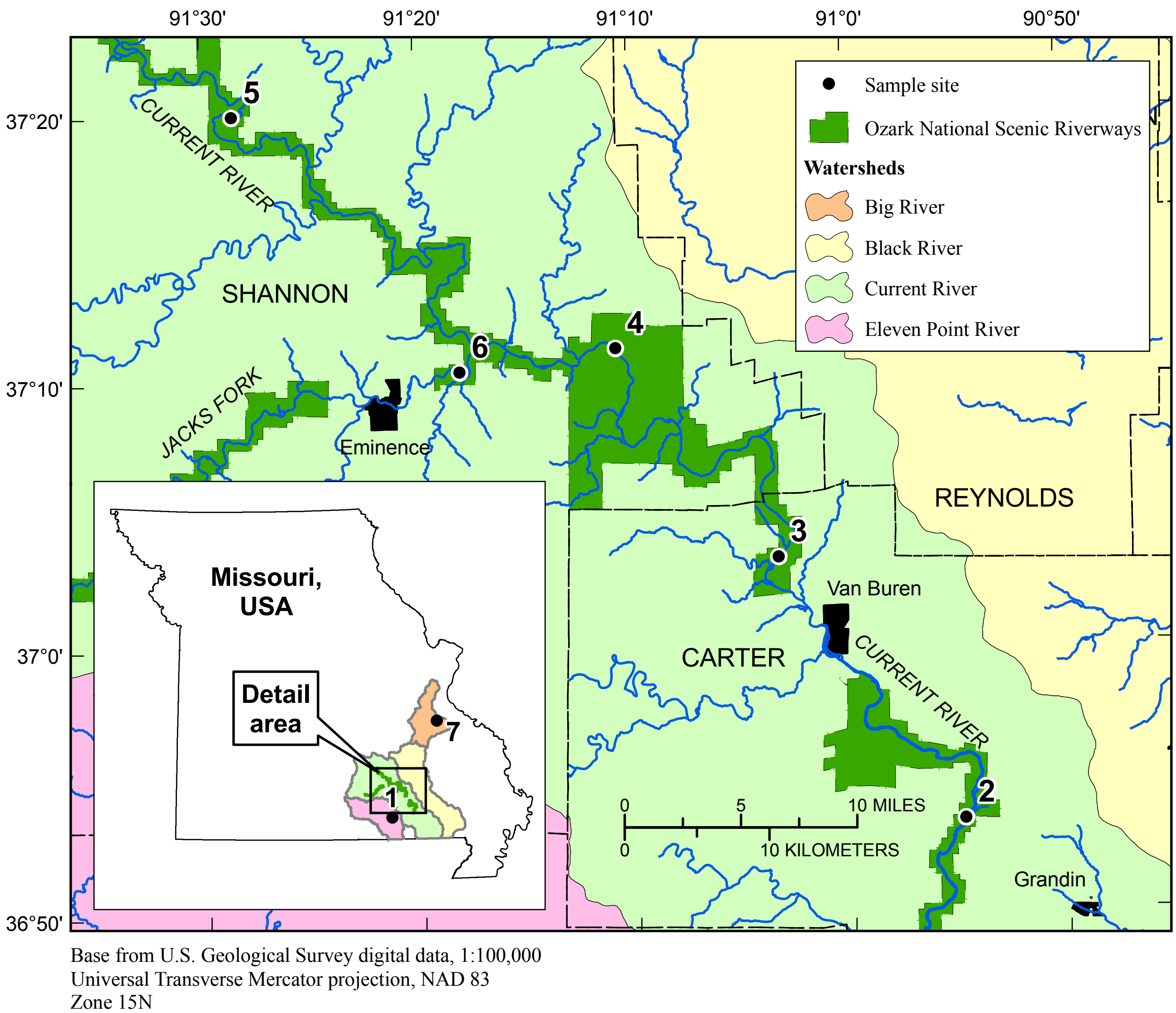

Figure 1. Location of the study area; shown are invertebrate collection sites (1 through 7), rivers, the Ozark National Scenic Riverways (ONSR) boundary, watershed boundaries, counties, and municipalities.

and discharges (Imes, 2002). Although lead does not accumulate to high concentrations in aquatic organisms, elevated concentrations and effects such as biochemical responses in fish and altered benthic fish and macro-invertebrate community composition have been associated with the release of metals from the Old Lead Belt and New Lead Belt (Schmitt and Finger, 1982, 1987; Whelan, 1983; Schmitt and others, 1984, 1987, 1992, 1993, 2005, 2006, 2007a, 2007b; Dwyer and others, 1988; Gale and others, 2004; Brumbaugh and others, 2005, 2007; Allert and others, 2006; Besser and others, 2007). ONSR managers have expressed a need to define and track concentrations of lead and possibly other potentially toxic metals out of concern for human and ecological health. These needs may become more acute if additional economically significant lead-zinc deposits are located and exploited.

Purpose and Scope

The U.S. Geological Survey (USGS) conducted a study in cooperation with the NPS to determine metals concentrations in invertebrates obtained in 2005 from streams in the ONSR as a pilot for a long-term monitoring program. The National Parks Omnibus Management Act of 1998 and NPS policy require that park managers know the condition of natural resources under their stewardship, and monitor long-term trends in those resources to fulfill the agency mission of conserving parks unimpaired. Accordingly, a comprehensive monitoring plan is being developed for the ONSR to assess its condition. Among the indicators being considered for inclusion in the monitoring plan are concentrations of lead and other potentially toxic metals released to aquatic ecosystems by lead-zinc mining. Monitoring will consist of measuring concentrations of metals in samples collected periodically from waters of the ONSR, with the objectives of determining current (2005) concentrations of lead and other metals in the rivers of the ONSR, whether concentrations are increasing, whether aquatic organisms are exposed to potentially toxic concentrations of lead or other metals, and whether individuals and populations of metal-sensitive river biota are potentially being affected by exposure to metals. This report summarizes the 2005 concentrations and thereby provides a basis for achieving the other monitoring objectives. Data from other studies (Petersen and others, 1998; Allert and others, 2006; Besser and others, 2007) are included for comparison.

Species Selection Criteria

Metal concentrations can be monitored in samples representing virtually all living and non-living components of aquatic ecosystems (water, sediment, plants, and animals). Each sample type has advantages and disadvantages that differ among the parameters (in this example, metals) to be monitored. General criteria for selecting a sample type or types include spatial and temporal variability, analytical detection thresholds, costs, and the degree to which concentrations in a given matrix represent "environmental conditions", including site fidelity—the extent to which an animal represents the location from which it was collected. Effects of contaminant exposure at many levels of biological organization (biochemical, organ, organism, population, and community) also can be monitored; however, except for certain specific biochemical responses (Schmitt and others, 1984; 2007), most effects are not contaminant-specific and can be influenced by many factors in addition to chemical exposure.

Crawford and Luoma (1993) completed an extensive literature review pertaining to organic and inorganic contaminant monitoring in biota as part of the of the USGS National Water-Quality Assessment Program development (NAWQA; Hirsch and others, 1988). The following criteria were identified for choosing species for chemical analysis: Chemical concentrations in the organisms should be responsive to environmental exposure; uptake of contaminants by organisms should be rapid; concentrations in the organisms should be greater than those in water; the organisms must not be killed by exposure to low levels of the contaminants to be monitored; concentrations in organisms should vary little within a site; the organisms should be relatively sedentary so as to reflect concentrations in the collection locale; the organisms should be abundant and widespread in the study area to facilitate comparisons; the organisms should be sufficiently large, abundant, or both to provide adequate tissue for analysis; the organisms should be sufficiently long-lived to integrate exposure concentrations over at least several months; and the organisms should be easy to sample (Crawford and Luoma, 1993). An additional consideration of the NPS is to minimize the removal of aquatic organisms, especially those considered to be rare, recreationally significant, or ecologically significant.

A problem shared by all programs and protocols relying on the collection of indigenous organisms is that there are no truly ubiquitous large, long-lived, abundant, and sedentary species that accumulate all contaminants equally well. Of the organisms available for consideration in the ONSR, certain invertebrates meet most requirements for trace-metal monitoring. Most aquatic macroinvertebrates are comparatively sedentary, and generally they do not internally regulate metals as well as fish. Many invertebrates also ingest varying amounts of particulate material (and its contaminants) from the water column, bed sediment, or both depending on feeding guild (for example Goodyear and McNeill, 1999). Consequently, metals concentrations in invertebrates tend to reflect environmental concentrations comparatively well (Crawford and Luoma, 1993; Goodyear and McNeill, 1999). The NAWQA program considered sampling fish, invertebrates (mollusks, crayfish, aquatic insect larvae), and plants (attached algae, macroalgae, macrophytes), and ultimately adopted a step-down approach for trace metals depending on taxa available at a site. Organisms are sampled by NAWQA in the following order of preference: Asian clams, aquatic insects, target fish species (as identified by Crawford and Luoma, 1993), and aquatic plants. In the Ozark Plateaus Study Unit (Petersen and others, 1998), which included waters of the ONSR, Asian clams were collected and analyzed by NAWQA where the species could be found and several fishes (liver tissue; Crawford and Luoma, 1993) were sampled elsewhere; liver samples were obtained preferentially from longear sunfish (*Lepomis megalotis*) and, secondarily, from smallmouth bass (*Micropterus dolomieu*). Crayfish met all the monitoring criteria identified by NAWQA but were not selected because no information was available on the uptake and retention of organic chemicals by these animals (Crawford and Luoma, 1993). Although organic chemicals remain a priority for NAWQA, they are not presently an issue in the ONSR. Based on a discussion of these factors with NPS personnel, crayfish and Asian clams were selected for pilot monitoring in the ONSR. Attributes of these organisms for metals monitoring are summarized in the following sections.

Crayfish

Crayfish constitute a large percentage of the invertebrate biomass in Ozark streams (Hobbs, 1993; Momot, 1995; Rabeni and others, 1995; Whitledge and Rabeni, 1997; DiStefano, 2005). As such, they are considered "ecological dominants" (Simberloff, 1998) that shape the entire aquatic community. Crayfish are opportunistic ominvores that feed on varying proportions of fish, aquatic invertebrates, periphyton, and detritus during their life cycle (Hobbs, 1993; Momot, 1995; Whitledge and Rabeni, 1997; Parkyn and others, 2001). Crayfish process large quantities of organic material (including macrophytes, attached algae, and detritus) and represent a significant food source for smallmouth bass, other fishes, and riparian wildlife (Probst and others, 1984; Whitledge and Rabeni, 1997; DiStefano, 2005). Crayfish tend to accumulate non-essential metals such as lead and cadmium in proportion to exposure, but may be able to regulate copper and zinc (Gillespie and others, 1977; Dickson and others, 1979; Knowlton and others, 1983; Crawford and Luoma, 1993). Concentrations of lead in crayfish from mining-affected streams in southeast Missouri were highly correlated with those in sediments, but were lower by a factor of about five (Schmitt and Finger, 1982). Most metals in crayfish concentrate in the hepatopancreas, antennal (green) gland, exoskeleton, and digestive tract (Dickson and others, 1979; Roldan and Shivers, 1987; Crawford and Luoma, 1993); however, mercury also accumulates in muscle (Allard and Stokes, 1989). The NAWQA program considered dissecting the hepatopancreas from crayfish for analysis (Crawford and Luoma, 1993); however, metals in whole crayfish (including the contents of the digestive tract) represent the concentrations to which higher-level organisms are exposed (Schmitt and others, 2006). In addition, there is a large body of extant data describing metals concentrations in whole crayfish from streams representing the range of metals concentrations present in Missouri to which concentrations in crayfish from the ONSR can be compared (Wixson, 1978; Schmitt and Finger, 1982; Whelan, 1983; Allen and Wilson, 1992; Wildhaber and others, 1997; Allert and others, 2006; Besser and others, 2007). There also are uptake data from locally relevant controlled studies (Knowlton and others, 1983; Besser and Rabeni, 1987), and the analysis of metals in crayfish represents an important component of ongoing USGS studies related to lead-zinc mining in Missouri (Imes, 2002; Allert and others, 2006; Besser and others, 2007).

Riffle-dwelling crayfish of the genus *Orconectes*, which are distributed across the Ozark Plateau (Pflieger, 1996), were targeted for monitoring in the ONSR. Within the ONSR, the golden crayfish (*O. luteus*) is the prevalent species (Rabeni and others, 1995); it is also widespread in the Ozarks. Consequently, and even though it does not grow as large as some other species, *O. luteus* was the preferred species. The spothanded crayfish (*O. punctimanus*) was identified as the primary alternate species in the event that *O. luteus* was not available at a site. Two other crayfishes, the Ozark crayfish (*O. ozarkae*) and Hubbs' crayfish (*Cambarus hubbsi*), also occur in the ONSR and were identified as the alternate species of last resort.

Asian Clam

The Asian clam is an exotic species that has become established throughout much of North America. It is extremely invasive and is regarded as a threat to indigenous mussels and other native aquatic organisms (McMahon, 1983; Oesch, 1995). In 1992, when NAWQA sampled the Current River and Jacks Fork for contaminants in biota, the Asian clam was available only at the downstream-most site on the Current River at Van Buren (Petersen and others, 1998). Since 1992, the species has spread throughout most of the Current River and Jacks Fork within the ONSR (V. Grant, ONSR, oral commun., 2005).

The use of the Asian clam for contaminants monitoring has grown in popularity as the species has spread. As a result, and as noted by Crawford and Luoma (1993), protocols for monitoring with Asian clams are well documented (Graney and others, 1983; Foe and Knight, 1987; Leland and Scudder, 1990). Asian clams accumulate most metals in proportion to exposure, with the possible exception of zinc (Crawford and Luoma, 1993). Laboratory exposure studies indicate a concentration-dependent linear uptake of mercury and cadmium, but with a plateau effect for cadmium, and that concentrations persist for at least 30 days post-exposure (Inza and others, 1998). Studies with caged, transplanted Asian clams indicate that tissue concentrations may not reflect zinc gradients in water or sediment as well as those of cadmium (Baudrimont and others, 1999). Nevertheless, data from the Spring River basin (Missouri, Kansas, and Oklahoma) indicate that concentrations of lead, cadmium, and zinc in wild Asian clams are correlated with concentrations in sediment (Angelo and others, 2007). The Asian clam was, therefore, selected for monitoring in the ONSR.

Materials and Methods

Collection Sites

Crayfish and Asian clams were obtained at five ONSR sites and two sites outside the ONSR during late August-early October, 2005 (fig. 1; table 1). Sites were selected to represent a broad range of metals exposures in the ONSR and nearby areas and for ease of access. One outside site was on the Eleven Point River, in Oregon County, Missouri, downstream from the prospecting area. The other was on the Big River in St. Francois County, Missouri; the Big River has been contaminated by mine tailings from the Old Lead Belt (Gale and others, 2004; Brumbaugh and others, 2005; Schmitt and others, 2006) and a human consumption advisory has been issued because of elevated concentrations of lead in bottom-dwelling fish (Missouri Department of Health and Senior Services, 2006). Together with extant contemporary data (Petersen and others, 1998; Allert and others, 2006; Besser and others, 2007), the seven sites represent the range of metals concentrations likely to exist in streams in and near the park. Following completion of the pilot, it is likely that a probability-based sampling plan will be developed by the NPS to be implemented in concert with other ONSR monitoring components. The revised sampling plan will define the boundaries of the "populations" being sampled and will address issues such as site selection criteria; sampling frequency, timing, and replication; and the number and location of sampling sites. The monitoring protocols conform to the general guidelines of Oakley and others (2003) and to all pertinent guidelines

Table 1. Location of collection sites and sampling dates.

Site number	Location	County	Date	Lat, long[a]
1	Eleven Point River at Turner's Mill	Oregon	8/29/2005	36° 45' 56.7" N, 91° 16' 01.0" W
2	Current River at Cataract Landing	Carter	8/30/2005	36° 53' 22.2" N, 90° 54' 47.3" W
3	Current River at Waymeyer Landing	Carter	8/31/2005	37° 03' 15.1" N, 91° 03' 16.8" W
4	Current River at Powdermill Ferry	Shannon	9/6/2005	37° 11' 10.4" N, 91° 10' 15.4" W
5	Current River at Pulltite Landing	Shannon	9/7/2005	37° 20' 01.7" N, 91° 28' 29.9" W
6	Jacks Fork at Shawnee Creek	Shannon	9/8/2005	37° 10' 21.3" N, 91° 18' 00.6" W
7	Big River at St. Francois State Park	Washington	10/4/2005	37° 57' 23.7" N, 90° 32' 29.2" W

[a]WGS 84; obtained by Global Positioning System (GPS) receiver.

for the humane treatment of test organisms during culture and experimentation.

Field Methods

Crayfish were captured by kick-seining or kick-netting in riffles containing coarse substrate or along the margins of emergent vegetation beds. They were removed from the nets and kept alive in a plastic bucket lined with a polyethylene bag containing ambient water. Monitoring in the ONSR targeted young-of-the-year golden crayfish collected in late summer-early fall, by which time they generally have attained a carapace length of 12 to 18 mm (24 to 36 mm total body length; Muck and others, 2002). When about 15 crayfish of the appropriate size had been collected they were transported to shore. The identification (species) of each animal was confirmed and its carapace length (CL, from tip of rostrum to posterior edge of the cephalothorax) was measured to the nearest 0.1 mm using stainless steel calipers. The carapace length was recorded and each crayfish was carefully rinsed with ambient water and placed in a pre-cleaned, pre-labeled polyethylene jar. The collection target was three composite samples of 10 crayfish (one composite sample per jar) from each site. Remaining crayfish were released. To be consistent with previous studies (Besser and others, 2007) and to ensure that the samples represented the metals concentrations available for transferred to higher trophic levels, crayfish were not depurated (that is, held in ambient water to allow them to purge ingested particulate material). After sampling, the approximate center of the area of the stream from which each composite sample was obtained was determined with a global positioning system (GPS) receiver. Samples were frozen in dry ice in the field, then transferred to a freezer upon return to the laboratory and stored frozen (-20 °C) until thawed for analysis.

Asian clams were harvested from riffles and stream margins by hand or with dip nets. The target was three composite samples of 10 animals each from each site. As described for crayfish, Asian clams were held in a lined plastic bucket containing ambient water until 10 to 15 specimens of the targeted size range (20 to 25 mm maximum shell diameter) had been obtained. This size was selected for conformity with NAWQA protocol (Crawford and Luoma, 1993). The specimens were transferred to shore where as much external material (sediment, algae) as possible was removed by hand from the external surfaces of the shells and the identity was confirmed. Each specimen was measured with stainless steel calipers (maximum shell diameter). In contrast to crayfish and to also remain consistent with NAWQA protocol (Crawford and Luoma, 1993), the Asian clams were depurated by placing all the animals comprising a sample in a new polyethylene bag about one third full of ambient water. The bag was then filled with oxygen, sealed, and placed in another (sealed) bag containing a sample label. Remaining specimens were released. The labeled, double-bagged sample was transferred to a cooler containing ambient water, and the sampling process was repeated until three samples of 10 animals had been obtained from the site. The approximate center of each collection site was documented by GPS. The specimens remained in the bags of ambient water in the coolers for 24 hours, after which they were transferred to pre-labeled, pre-cleaned polyethylene jars (one composite sample per jar) and frozen (-20 °C) until thawed for analysis.

Laboratory Analyses and Quality Assurance

Analysis of invertebrate samples followed Crawford and Luoma (1993), May and others (1997), Brumbaugh and others (2005), and Besser and others (2007). Composite samples of whole crayfish (with intact exoskeleta and digestive tracts) were prepared for analysis by lyophilization followed by homogenization to a coarse powder with a cryogenic mill. Asian clams were partly thawed so that the soft tissues could be separated from the shells. The soft tissues of all the animals in the composite sample were lyophilized and homogenized by pulverizing them with a glass rod. Dried, homogenized samples were digested in nitric acid and hydrogen peroxide as described by Brumbaugh and others (2005). Digestates were analyzed for cobalt, lead, cadmium, zinc, and nickel by inductively coupled plasma mass spectrometry (Brumbaugh and others, 2005; Besser and others, 2007). All concentrations are reported as micrograms per gram (µg/g) dry-weight; moisture content was not determined.

Quality-control measures incorporated at the digestion stage of the analyses included digestion blanks, certified reference materials, replicates, and pre-and post-digestion fortified samples (spikes). A calibration blank and an independent calibration verification standard were analyzed every 10 samples to confirm the calibration status of the instrument throughout the analyses. Recoveries of the calibration standards were 91 to 108 percent for crayfish and 99 to 105 percent for Asian clams (appendix 1; appendices are at the end of the report). As a check for potential interferences, dilution percent differences (DPDs) based on five-fold dilutions of the sample digestates were determined; DPDs were less than eight percent for all analytes. Instrument and method detection limits (IDLs, MDLs) and method quantitation limits (MQLs) were estimated for each element in each matrix based on the standard deviations of the concentrations determined in blanks (appendix 1). The upper limits of the MQLs ranged from 0.017 µg/g dry weight for cadmium to 0.63 µg/g dry weight for zinc (appendix 1). Instrumental precision, which was determined as relative percent difference (RPD) between duplicate analyses of digestates, was 0.1 to 4.8 percent (appendix 1). Recoveries of metals from pre-digestion spikes carried through the entire analytical procedure were 78 to 107 percent in crayfish and 85 to 112 percent in Asian clams, whereas recoveries of elements spiked into digestates were 81 to 106 percent in crayfish and 83 to 105 percent in Asian clams (appendix 1). Relative standard deviations (RSDs) of triplicate determinations representing the entire procedure (digestion through instrumental analysis) indicated a low degree of variability for crayfish, with RSDs ranging from 2.4 percent for zinc to 7.2 percent for nickel. The Asian clams, which were not cryogenically homogenized, were less replicable; RSDs ranged from 8.3 percent for cobalt to 24 percent for lead (appendix 1). Recoveries of metals from National Institute of Standards (NIST) reference materials (oyster tissue) carried through the entire procedure were within or near accepted limits for most analytes, the notable exceptions being slightly lower than expected recoveries of lead (as low as 87 percent) in some analyses (appendix 2). Overall, the quality control analyses indicate that the results reported here accurately represent the metals concentrations in crayfish and Asian clams.

Data Analysis and Presentation

Summary statistics describing sample composition, organism size, and metals concentrations are presented in tabular and graphical formats. No statistical analyses were performed and only limited interpretation of the findings is provided. Raw data describing the exact collection locations and the sizes of the animals in each sample are tabulated in appendices 3 and 4.

Results

Crayfish

A total of 193 crayfish (22 composite samples) representing the seven sites were collected and analyzed, but the animals were not all of the same species or size. Golden crayfish were obtained at all five sites within the ONSR, where all samples except one comprised 10 animals (table 2). Golden crayfish were not present at site 1 (Eleven Point River), and at site 7 (Big River) they were less abundant than at the ONSR sites; only three golden crayfish were obtained at site 7. The other samples from site 7 were belted crayfish (*O. harrisoni*), a species known only from the Meramec River system in Missouri (Pflieger, 1996), and spothanded crayfish (table 2). Site 1 yielded exclusively coldwater crayfish (*O. eupunctus*), a species restricted in Missouri to the Eleven Point River system (Pflieger, 1996).

Mean carapace length of golden crayfish was comparatively uniform among the sites, ranging from 17.9 mm in one sample from site 3 to 23.3 mm at site 5 (table 2). Nevertheless, the golden crayfish from sites 4 and 5 were larger generally than those from sites 2, 3, 6, and 7 (table 2). Within-sample coefficients of variation (CVs) for golden crayfish carapace length were 7.8 to 20.2 percent, the latter at site 2. Relative to the sample means, carapace length ranges were typically 20 to 40 percent of the mean, with some exceeding 50 percent (table 2). Coldwater crayfish were obtained exclusively at site 1. On average the coldwater crayfish were about the same size as the golden crayfish from sites 2 through 7, but more uniform (table 2). Two samples of spothanded crayfish and one of belted crayfish, each comprising three to five animals, were also obtained from site 7. Although the belted crayfish is unique to the Meramec River system, it is similar in most regards to other species of *Orconectes* (Pflieger, 1996). The animals in one spothanded crayfish sample from site 7 were larger (mean carapace length = 35.9 mm) than other samples analyzed, whereas those in the second sample were about the same size as the other species (table 2). The belted crayfish also were about the same size as most golden crayfish. Additional data pertaining to crayfish size and the location from which each sample was obtained are tabulated in appendix 3.

All five metals were detected in all crayfish samples. Concentrations (all µg/g dry weight) were cobalt, 0.69 to 4.15; nickel, 0.61 to 4.93; zinc, 58.3 to 179; cadmium, 0.25 to 1.23; and lead, 0.11 to 143 (table 2). All maximum concentrations were in samples from site 7 (Big River) and all minima were in samples from within the ONSR (table 2). Site means (all µg/g dry weight) were cobalt, 0.73 to 3.06; nickel, 0.64 to 3.89; zinc, 63.3 to 140; cadmium, 0.29 to 1.02; and lead, 0.14 to 94.0 (tables 2 and 3; fig. 2). The site means followed the same spatial pattern as the maxima. These minimum and maximum mean concentrations differ by factors of 4.2 for cobalt, 6.1 for nickel, 2.2 for zinc, 3.5 for cadmium, and 671 for lead. Within-site CVs for sites 1 through 6 were typically less than

Table 2. Species, numbers of specimens, carapace lengths, and concentrations of metals in composite samples of crayfish (*Orconectes* spp.) from the indicated sites.

[*n*, number of specimens; Min, minimum; Max, maximum; CV, coefficient of variation; Percent, range as a percentage of the mean; OE, coldwater crayfish, *O. eupunctus;* OL, golden crayfish, *Orconectes luteus*; OP, spothanded crayfish, *O. punctimanus*; OH, belted crayfish, *O. harrisoni*; all concentrations in µg/g dry weight]

			Carapace length						Metal				
Sample	Species	*n*	Mean	Min	Max	CV	Range	Percent	Cobalt	Nickel	Zinc	Cadmium	Lead
Site 1, Eleven Point River at Turner's Mill													
A	OE	10	22.4	19.1	25.3	8.0	6.2	27.6	1.20	1.61	88.1	0.52	0.34
B	OE	10	21.2	19.3	23.4	6.7	4.1	19.3	1.32	1.28	73.5	0.44	0.20
C	OE	10	21.9	17.1	25.0	10.8	7.9	36.2	1.23	1.47	73.0	0.44	0.26
Site 2, Current River at Cataract Landing													
A	OL	10	18.9	12.5	24.3	20.2	11.8	62.6	1.02	1.07	84.9	0.48	0.37
B	OL	8	20.7	15.9	26.4	18.1	10.5	50.8	0.77	0.81	76.3	0.60	0.20
C	OL	10	20.2	17.2	27.8	17.1	10.6	52.5	0.76	0.84	76.7	0.70	0.16
Site 3, Current River at Waymeyer Landing													
A	OL	10	17.9	15.5	23.3	11.8	7.8	43.7	0.76	0.79	69.3	0.77	0.16
B	OL	10	19.2	17.0	25.3	15.2	8.3	43.3	0.70	0.74	65.3	0.70	0.14
C	OL	10	19.0	17.0	24.4	11.9	7.4	38.9	0.74	0.72	62.6	0.61	0.18
Site 4, Current River at Powdermill Ferry													
A	OL	10	20.1	17.8	24.4	11.5	6.6	32.6	0.79	0.61	66.4	0.38	0.11
B	OL	10	22.4	20.3	25.3	7.8	4.9	21.9	0.70	0.71	78.0	0.50	0.18
C	OL	10	20.3	17.1	23.7	9.8	6.6	32.2	0.69	0.61	61.9	0.28	0.12
Site 5, Current River at Pulltite Landing													
A	OL	10	21.9	17.4	25.5	12.3	8.1	37.1	0.71	0.78	64.4	0.32	0.16
B	OL	10	23.3	20.0	26.2	8.8	6.2	26.7	0.89	1.10	58.3	0.29	0.14
C	OL	10	20.7	18.0	23.9	10.3	5.9	28.5	1.03	1.25	67.1	0.29	0.20
Site 6, Jacks Fork at Shawnee Creek													
A	OL	10	19.0	15.3	22.9	16.5	7.6	40.0	1.27	1.19	71.4	0.33	0.26
B	OL	10	18.6	16.4	22.8	13.8	6.4	34.4	1.14	1.05	65.9	0.25	0.17
C	OL	10	19.9	15.4	26.7	18.2	11.3	56.9	1.14	0.87	65.1	0.30	0.15
Site 7, Big River at St. Francois State Park													
A	OP	5	35.9	33.4	37.8	4.9	4.4	12.2	2.87	3.57	108.	0.65	71.3
B	OH	3	24.1	20.9	27.4	13.5	6.5	27.0	4.15	4.93	144.	1.09	143.
C	OP	4	24.7	15.9	30.2	25.0	14.3	58.0	3.04	3.93	179.	1.09	85.0
D	OL	3	20.3	17.5	22.3	12.4	4.8	23.6	2.17	3.12	128.	1.23	76.6

Table 3. Mean concentrations of metals in composite samples of crayfish (*Orconectes* spp.) from the indicated sites.

[See table 1 for site locations; *n*, number of composite samples; OE, coldwater crayfish, *Orconectes eupunctus*; OL, golden crayfish, *O. luteus*; OP, spothanded crayfish, *O. punctimanus*; OH, belted crayfish, *O. harrisoni*; numbers in parentheses are coefficients of variation; all concentrations in µg/g dry weight]

Site number, species	*n*	Cobalt		Nickel		Zinc		Cadmium		Lead	
1, OE	3	1.25	(5.0)	1.45	(11.0)	78.2	(11.0)	0.47	(9.9)	0.27	(26.0)
2, OL	3	0.85	(17.0)	0.91	(16.0)	79.3	(6.1)	0.59	(19.0)	0.24	(46.0)
3, OL	3	0.73	(4.2)	0.75	(4.8)	65.7	(5.1)	0.69	(12.0)	0.16	(13.0)
4, OL	3	0.73	(7.6)	0.64	(9.0)	68.8	(12.0)	0.39	(29.0)	0.14	(28.0)
5, OL	3	0.88	(18.0)	1.04	(23.0)	63.3	(7.1)	0.30	(5.8)	0.17	(18.0)
6, OL	3	1.18	(6.3)	1.04	(15.0)	67.5	(5.0)	0.29	(14.0)	0.19	(30.0)
7, OL, OP, OH	4	3.06	(23.0)	3.89	(18.0)	140.	(25.0)	1.02	(25.0)	94.0	(40.0)

20 percent for all metals except lead, for which some exceeded 25 percent (table 3). Most CVs for site 7 were greater than those for the other sites, possibly reflecting species, size, and habitat differences at this site.

Mean concentrations of lead, zinc, and cadmium in crayfish from sites 1 through 6 (Current River, Jacks Fork, and Eleven Point River) were within the range of site means for reference sites reported by Allert and others (2006) and Besser and others (2007; fig . 3; table 4). Mean concentrations of lead and zinc, but not of cadmium, in crayfish from site 7 exceeded the site means for crayfish from New Lead Belt mining sites (tables 2, 3 and 4) but are similar to previously reported concentrations in crayfish from the Big River (Schmitt and others, 2006). Concentrations of lead and zinc in crayfish from sites 1 through 6 also were similar to those from Big Spring and Blue Spring, which contribute flow to the Current River; and in crayfish from Greer Spring, which contributes substantially to the flow in the Eleven Point River upstream from site 1 (fig. 3). Metals concentrations in crayfish from the Eleven Point River at Cane Bluff, which is upstream from Greer Spring, and from the mouth of Hurricane Creek, a tributary of the Eleven Point River just upstream from site 1, were greater than all others except the Big River; however, lead concentrations in crayfish from the Eleven Point River near McCormack Lake, which is just upstream from Greer Spring, were similar to those from other sites on the Eleven Point River, Current River, and Jacks Fork (fig. 3). Concentrations of cadmium in crayfish from Greer Spring and Blue Spring, as reported by Besser and others (2007), were also greater than those from all other non-mining sites (fig. 3). Mean cobalt concentrations in crayfish from sites 1 through 6 were within the range for reference sites reported by Allert and others (2006), but mean nickel concentrations at sites 1, 2, 5, and 6 exceeded the reference range. Concentrations of cobalt and nickel were about 30-fold greater in crayfish from one New Lead Belt site (Strother Creek) investigated by Allert and others (2006) than at reference sites (table 4). It also is important to note that crayfish could not be obtained by Allert and others (2006) or Besser and others (2007) at one site further upstream on Strother Creek, where elevated concentrations of cobalt and nickel were reported in fish (Schmitt and others, 2007a).

Asian Clam

A total of 202 Asian clams (21 composite samples) representing all seven sites were collected and analyzed. Most samples contained 10 animals, but those from site 1 contained only seven or eight each (table 5).

The specimens were smaller than the preferred range, but of similar size (mean diameter 15.5 to 21.4 mm) at sites 1 through 6; those from site 7 were smaller still (10.9 to 13.4 mm; table 5). Within-sample CVs for shell diameter were 4.3 to 18 percent, the latter in a sample from site 3 for which the size range as a percentage of the mean was 55.3 percent (table 5). Additional data pertaining to Asian clam size and the location from which each sample was obtained are tabulated in appendix 4.

As was true for crayfish, all five metals were detected in all Asian clam samples. One sample from site 5 contained 7.83 µg/g dry weight of cadmium, 1.45 µg/g cobalt, 1.27 µg/g nickel, 178 µg/g zinc, and 0.31 µg/g lead. The cadmium concentration in this sample was about seven times greater than all other samples not from the Big River. The sample was re-analyzed; the concentration was 1.10 µg/g, which was within the range of the other samples not from the Big River, and 1.29 µg/g cobalt, 1.19 µg/g nickel, 138 µg/g zinc, and 0.32 µg/g lead. These results indicate that the first sub-sample had been contaminated by cadmium; therefore, the second set of values is reported. With this substitution, concentration ranges for Asian clams (all µg/g dry weight) were cobalt, 1.16 to 2.00; nickel, 0.65 to 11.3; zinc, 129 to 812; cadmium, 0.98 to 26.5; and lead, 0.15 to 85.8 (table 5). Maximum concentrations of all metals except cobalt were in samples from site 7

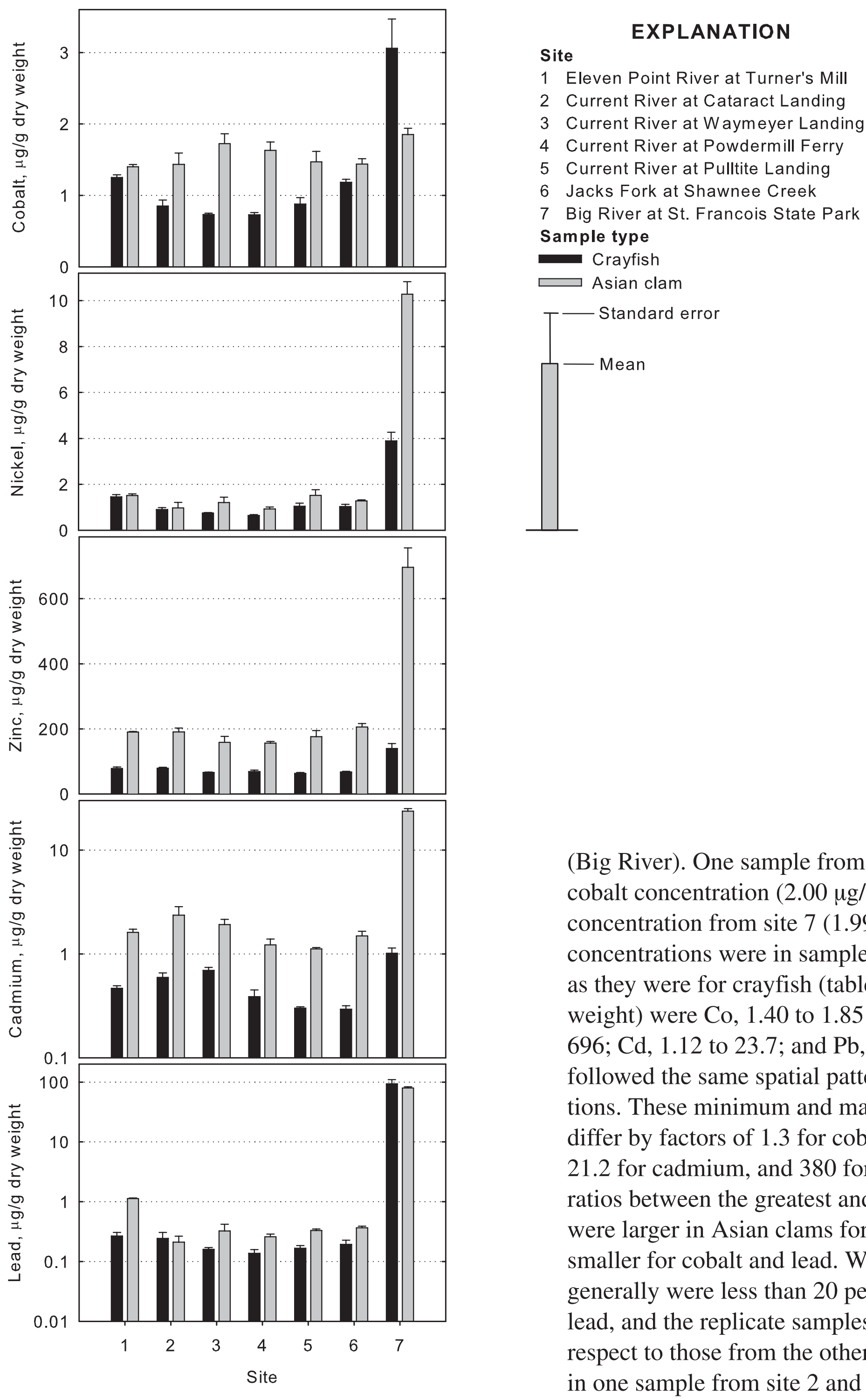

Figure 2. Concentrations of cobalt, nickel, zinc, cadmium, and lead in crayfish and Asian clams from sites sampled in 2005.

(Big River). One sample from site 3 contained about the same cobalt concentration (2.00 µg/g dry weight) as the greatest concentration from site 7 (1.99 µg/g; table 5). All minimum concentrations were in samples from sites within the ONSR, as they were for crayfish (table 5). Site means (all µg/g dry weight) were Co, 1.40 to 1.85; Ni, 0.93 to 10.38; Zn, 156 to 696; Cd, 1.12 to 23.7; and Pb, 0.21 to 79.8 (table 6; fig. 2) and followed the same spatial pattern as the maximum concentrations. These minimum and maximum mean concentrations differ by factors of 1.3 for cobalt, 11.1 for nickel, 4.5 for zinc, 21.2 for cadmium, and 380 for lead. Relative to crayfish, the ratios between the greatest and smallest mean concentrations were larger in Asian clams for nickel, zinc, and cadmium, but smaller for cobalt and lead. Within-site CVs for Asian clams generally were less than 20 percent for all metals, including lead, and the replicate samples from site 7 were similar in this respect to those from the other sites (table 6). The CV for Ni in one sample from site 2 and one for Pb from each of sites 2 and 3 were larger than most (greater than 40%; table 6).

Concentrations of metals in Asian clams were similar to those reported for the same or equivalent streams across the Ozarks by Petersen and others (1998; table 7). Site 2 was downstream of the Current River at Van Buren NAWQA site, and site 7 was upstream of the Big River at Richwoods NAWQA site. Metals concentrations in Asian clams from these sites agreed well with the NAWQA data (tables 6 and 7).

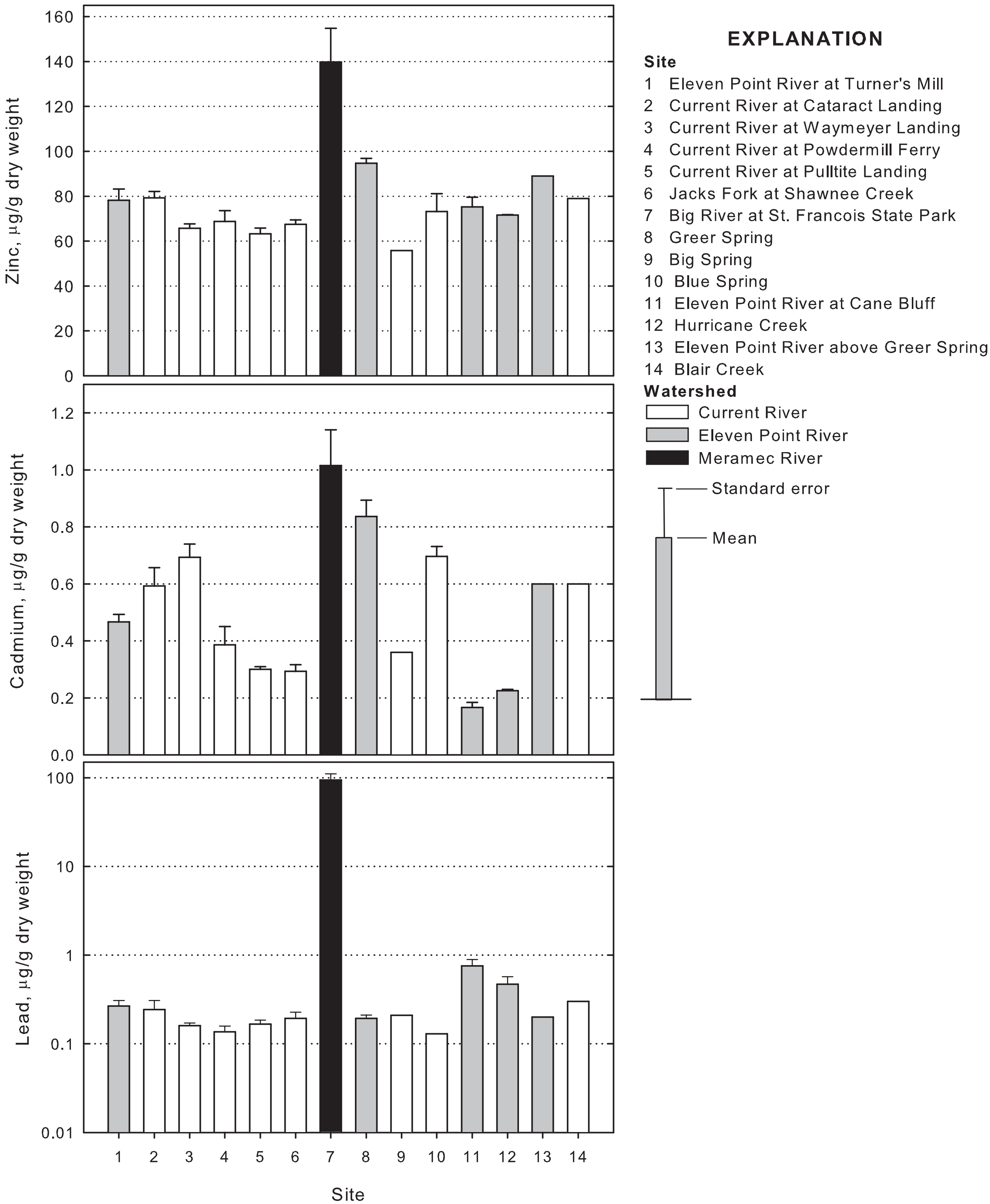

Figure 3. Concentrations of zinc, cadmium, and lead in composite samples of crayfish from sites in three watersheds sampled in 2005 (sites 1 through 7), in 2001–02 by Besser and others (2007; sites 8 through 12), and in 2004 by Allert and others (2006; sites 13 and 14).

Table 4. Concentrations of metals in crayfish (*Orconectes* spp.) obtained in 2001 by Besser and others (2007) and in 2004–05 by Allert and others (2006).

[Mining sites were located less than 10 km downstream from mines in the New Lead Belt, downstream sites were located greater than 10 km downstream from mines, and there was no mining upstream from reference sites; *n*, number of sites; all concentrations in µg/g dry weight; nd, not determined]

Site type	Lead	Zinc	Cadmium	Nickel	Cobalt
Besser and others (2007)[a]					
Mining (*n* = 6)[b]	1.5–16.0	94.0–121	0.32–5.10	nd	nd
Downstream (*n* = 4)	0.5–2.5	77.0–90.0	0.22–0.65	nd	nd
Reference (*n* = 5)[c]	0.2–1.3	72.0–84.0	0.17–0.84	nd	nd
Allert and others (2006)[c]					
Mining (*n* = 3)[b]	4.0–18.0	94.0–100	1.1–2.0	1.6–6.6	1.5–3.5
Downstream (*n* = 9)	0.8–4.0	83.0–160	0.6–5.9	0.7–19.0	0.6–24.0
Reference (*n* = 4)	0.2–0.4	77.0–89.0	0.4–0.8	0.6–0.8	0.8–1.4

[a]Ranges of site means based on 3 composite samples of 7 to 10 animals per site.

[b]Crayfish absent at one mining site.

[c]One composite sample per site.

Discussion

Based on a review of the monitoring literature (Crawford and Luoma, 1993), we sought young-of-the-year golden crayfish of 12 to 18 mm carapace length and Asian clams of 20 to 25 mm maximum shell diameter for monitoring. Crayfish were abundant at sites 1 through 6, but were difficult to locate at site 7 (Big River). Golden crayfish was the dominant species only in the Current River and Jacks Fork. Crayfish of all species from all sites generally were larger than 18 mm carapace length by early September, and the preferred size range was not available. Because crayfish molt several times during the growing season, which may affect metals concentrations, samples were collected as consistently as possible with respect to sampling period and size. However, reports of size effects on metals concentrations vary; for example, Knowlton and others (1983) indicated that lead concentrations decreased with size, but Dickson and others (1979) reported no size-related concentration effects.

Although present at all sites, Asian clams were more difficult to locate than crayfish. At most sites they typically were less than 20 mm in diameter, which is smaller than the targeted size range (20 to 25 mm). In the Spring River system, cadmium concentrations in the soft tissues of un-depurated Asian clams from a relatively uncontaminated site increased with shell diameter, lead concentrations decreased, and there was no trend for zinc (Angelo and others, 2007). Mean shell diameters reported by Angelo and others (2007) ranged from smaller than 15 mm to greater than 25.1 mm, which is similar to the range in this study. Angelo and others (2007) noted that when considered across the sites they investigated, the greatest metals concentrations tended to occur at sites dominated by small Asian clams; the effects of collection site and animal size could not be separated. Angelo and others (2007) also reported that concentrations of Pb, but not Cd or Zn, declined after depuration for 24 hours. Because concentrations of aluminum, an important constituent of sediment, also declined, the authors concluded that the reduction in the lead concentration was due to the egestion of sediment particles. Consequently, the size effects reported for lead by Angelo and others (2007) were also influenced to some degree by ingested sediment differences.

Despite the range of animal sizes, the concentrations of the metals measured in crayfish and Asian clams were within the ranges reported by other studies. Therefore, it appears that the target size range for crayfish could be increased in future collections. Based on our sampling, sufficient numbers of golden crayfish of 15 to 28 mm carapace length should be present in most of the Current River and Jacks Fork within the ONSR. Although Asian clams of 12 to 25 mm shell diameter also may be present in much of the ONSR, this species is more sparsely distributed, and might not be present at all potential sampling locations. In addition, the increasing concentrations of cadmium with shell diameter from a relatively uncontaminated site reported by Angelo and others (2007) indicates that size uniformity should be the goal of monitoring with Asian clams. Absent uniformity, investigators should remain cognizant of potential size effects on cadmium concentrations when interpreting the data. Moreover, neither crayfish nor Asian clams were homogeneously distributed at most sites. Crayfish could be obtained by kick-netting in riffles at most sites, but at site 7 they had to be dislodged from vegetation. Asian clams typically were located in discrete patches along stream margins at sites 2 through 6 (Current River and Jacks Fork), but at site 1 (Eleven Point River) they were located only in a backwater, and at site 7 (Big River) they were dispersed across the stream channel. The combined effects of

Table 5. Numbers of specimens, shell diameters, and concentrations of metals in composite samples of Asian clams from the indicated sites.

[*n*, number of specimens; Min, minimum; Max, maximum; CV, coefficient of variation; Percent, range as a percentage of the mean; all concentrations in µg/g dry weight]

Sample	*n*	Shell diameter						Metal				
		Mean	Min	Max	CV	Range	Percent	Cobalt	Nickel	Zinc	Cadmium	Lead
Site 1, Eleven Point River at Turner's Mill												
A	8	18.7	13.8	22.3	8.5	45.6	17.0	1.36	1.39	187	1.39	1.09
B	7	20.4	17.4	22.7	5.3	25.9	7.8	1.38	1.55	191	1.77	1.14
C	7	21.1	19.1	25.1	6.0	28.5	11.3	1.46	1.61	193	1.68	1.17
Site 2, Current River at Cataract Landing												
A	10	17.4	13.8	22.5	8.7	50.1	14.7	1.16	0.84	207	1.88	0.16
B	10	21.4	20.0	23.7	3.7	17.3	4.9	1.43	0.65	197	1.86	0.15
C	10	21.0	19.3	22.2	2.9	13.8	4.7	1.71	1.44	168	3.34	0.32
Site 3, Current River at Waymeyer Landing												
A	10	19.4	15.6	26.3	10.7	55.3	18.0	2.00	1.50	129	2.37	0.51
B	10	18.3	16.4	24.0	7.6	41.6	11.9	1.61	1.38	155	1.59	0.19
C	10	18.2	15.7	22.3	6.6	36.2	12.1	1.56	0.74	192	1.79	0.27
Site 4, Current River at Powdermill Ferry												
A	10	16.7	14.8	22.7	7.9	47.4	13.2	1.62	0.82	167	1.54	0.22
B	10	15.6	12.7	20.0	7.3	46.8	14.9	1.84	1.09	151	0.98	0.31
C	10	15.5	13.2	18.5	5.3	34.2	11.6	1.43	0.88	150	1.15	0.25
Site 5, Current River at Pulltite Landing												
A	10	18.3	16.4	22.8	6.4	35.0	10.0	1.76	2.00	202	1.18	0.37
B[a]	10	18.4	17.7	20.1	2.4	13.0	4.3	1.29	1.19	138	1.10	0.32
C	10	16.6	15.0	17.7	2.7	16.3	5.2	1.36	1.37	187	1.07	0.30
Site 6, Jacks Fork at Shawnee Creek												
A	10	16.7	14.2	20.4	6.2	37.1	9.7	1.32	1.21	193	1.45	0.36
B	10	18.0	16.0	21.3	5.3	29.5	9.7	1.41	1.36	199	1.79	0.33
C	10	16.9	14.9	18.6	3.7	21.9	6.6	1.58	1.27	226	1.23	0.41
Site 7, Big River at St. Francois State Park												
A	10	13.4	12.2	14.9	2.7	20.1	7.2	1.68	9.44	812	22.1	73.3
B	10	11.6	10.3	12.6	2.3	19.8	5.8	1.99	11.30	614	26.5	85.8
C	10	10.9	9.4	12.7	3.3	30.4	11.0	1.88	10.10	663	22.5	80.3

[a]Re-analyzed; original analysis was 7.83 µg/g cadmium, 1.45 µg/g cobalt, 1.27 µg/g nickel, 178 µg/g zinc, and 0.31 µg/g lead.

Table 6. Mean concentrations of metals in composite samples of Asian clams from the indicated sites.

[See table 1 for site locations; means of three composite samples; numbers in parentheses are coefficients of variation; all concentrations in µg/g dry weight]

Site number	Cobalt		Nickel		Zinc		Cadmium		Lead	
1	1.40	(3.6)	1.52	(7.3)	190	(1.6)	1.61	(12.0)	1.13	(3.5)
2	1.43	(19.0)	0.98	(41.0)	191	(11.0)	2.36	(32.0)	0.21	(41.0)
3	1.72	(13.0)	1.21	(32.0)	159	(20.0)	1.92	(20.0)	0.32	(50.0)
4	1.63	(13.0)	0.93	(15.0)	156	(5.5)	1.22	(23.0)	0.26	(17.0)
5	1.47	(16.0)	1.52	(27.0)	176	(18.0)	1.12	(4.9)	0.33	(11.0)
6	1.44	(9.1)	1.28	(5.9)	206	(8.1)	1.49	(19.0)	0.37	(11.0)
7	1.85	(8.4)	10.3	(9.1)	696	(14.0)	23.7	(9.4)	79.8	(7.8)

Table 7. Concentrations of metals in Asian clams obtained from the indicated Ozark Plateau sites by the USGS National Water Quality Assessment Program (NAWQA) during 1992–95.

[*n*, number of samples; all concentrations in µg/g dry weight; <, less than; NAWQA data summarized by Petersen and others (1998), available online at *http://infotrek.er.usgs.gov/traverse/f?p=NAWQA:HOME:1183417128461336*]

Site (*n*)	Cobalt	Nickel	Zinc	Cadmium	Lead
Niangua River at Windyville, Mo. (3)	1.4–2.0	1.4–2.3	131–189	0.8–1.1	<0.6-0.6
Osage River near St. Thomas, Mo. (1)	1.3	2.6	113	0.9	<0.4
Big River near Richwoods, Mo. (1)	3.5	7.6	514	19.3	134
Meramec River near Eureka, Mo. (1)	2.1	3.2	296	2.2	12.2
Kings River near Berryville, Ark. (2)	2.4–3.4	2.3–3.8	227–230	1.0–1.6	<0.5–<0.8
James River near Boaz, Mo. (1)	1.1	1.9	222	1.5	1.0
Buffalo River near St. Joe, Ark. (1)	1.3	2.1	165	1.7	<0.7
Black River near Lesterville, Mo. (1)	2.5	5.2	131	2.4	3.1
Current River at Van Buren, Mo. (1)	1.5	1.8	187	1.6	<0.6
Strawberry River north of Poughkeepsie, Ark. (2)	2.0–2.0	1.7–1.9	167–172	0.8–0.9	0.5–0.5
Neosho River near Parsons, Kans. (1)	0.7	1.9	135	1.3	<0.3
Elk River near Tiff City, Mo. (1)	<0.8	1.3	187	2.4	<0.8

such size, location, and habitat differences on metals concentrations are largely unknown for either crayfish or Asian clams.

The CVs for cadmium, zinc, and cobalt were similar for Asian clams and crayfish, but those for lead and nickel were greater in Asian clams (tables 3 and 6). The greater CVs for lead and nickel in Asian clams were somewhat unexpected considering that the crayfish were analyzed whole and only the soft tissues of the Asian clams were analyzed. However, the crayfish were homogenized with a cryogenic mill before digestion whereas the Asian clams were not because of small sample mass. These results indicate that as processed, nickel and lead were less homogeneously distributed in the soft tissues of Asian clams than in the whole crayfish. Future sampling might, therefore, call for a larger number of Asian clams per composite sample to ensure sufficient mass for cryogenic homogenization, or the use of cryogenic grinding equipment that is more suitable for small tissue masses.

Concentrations of all five metals in crayfish and Asian clams were greatest in samples from site 7 (Big River) and smallest at one or more sites within the ONSR, as expected. Concentrations of zinc and cadmium were uniformly greater in Asian clams than in crayfish; however, the other metals were variable and the patterns were not identical in the two sets of samples (fig. 2). Concentrations of zinc in crayfish were nearly uniform across the seven sites, supporting the generally held belief that this essential element is regulated by crayfish (Alikhan and others, 1990). Conversely, the much greater concentrations of zinc in Asian clams than in crayfish from site 7 indicate that zinc is not regulated to the same extent by Asian clams. The converse was true for cobalt, which is also an essential element; concentrations in Asian clams were more or less uniform across the seven sites, whereas concentrations in crayfish differed among sites and were substantially greater at site 7 than elsewhere (fig. 2). Allert and others (2006) also reported that cobalt, along with nickel, is accumulated by crayfish (table 5). Collectively, these findings indicate that the internal dynamics of the metals differ between the two taxa, and that neither is clearly superior to the other as a monitoring organism for all five metals; in other words, the two sets of samples provide different information. The elevated concentrations of cobalt, nickel, and other metals, in addition to lead, in biota from streams draining mining areas also highlights the fact that pollutants beyond lead may be released to the ONSR by lead-zinc mining.

This pilot investigation addressed only the ONSR objective of documenting current metals concentrations. Continued monitoring, combined with data from other aspects of the park-wide monitoring plan, should provide information for the remaining objectives. Future research should investigate the effects of organism size, age, gender, habitat (including temperature), species, and other factors on metals concentrations, and should seek to optimize the collection protocols and sampling design for long-term monitoring.

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Appendixes 1–4

Appendix 1. Instrument detection limits, method detection limits (MDLs)[a], method quantitation limits (MQLs)[b], sample and digestate replicate precision, and pre- and post-digestion spike recoveries for analyses of metals in crayfish and Asian clams.

[Instrument detection limit, ng/mL; method detection and quantitiation limits, µg/g dry weight]

Metal	Instrument detection limits	Method detection limits	Method quantitation limits	Sample replicate precision (percent)[c]	Digestate replicate precision (percent)[d]	Pre-digestion spike recovery (percent)[d]	Post-digestion spike recovery (percent)[e]	Calibration blank and standard recovery (percent)[f]
				Crayfish				
Cobalt	0.001	0.006	0.020	4.2	0.2–2.2	85–86	88–99	93–105
Nickel	0.015	0.030	0.099	7.2	0.4–2.4	80–86	84–94	91–104
Zinc	3.58	0.190	0.630	2.4	1.0–3.1	78–81	81–89	90–108
Cadmium	0.003	0.005	0.017	4.6	0.1	105–107	104–106	101–103
Lead	0.005	0.010	0.033	3.9	0.5–1.5	91–96	99	100–102
				Asian clams				
Cobalt	0.001	0.006	0.020	8.3	0.3–0.4	96–97	90–97	101–105
Nickel	0.012	0.029	0.096	19.0	0.5–1.5	97–98	88–98	99–105
Zinc	3.58	0.160	0.530	7.0	0.6–4.8	85–95	83–93	100–104
Cadmium	0.003	0.010	0.033	8.5	1.0–2.1	104–112	98–105	100–110
Lead	0.005	0.047	0.160	24.0	0.6–0.8	99–101	94–102	101–104

[a]MDL = $3 \times (SD_b^2 + SD_s^2)^{1/2}$, where SD_b = standard deviation of digestion blanks (n = 3) and SD^s = standard deviation of a low level standard diluted 100 × (n = 3).

[b]MQL = 3.3 × MDL.

[c]$n = 3$.

[d]$n = 2$.

[e]$n = 2$ for crayfish, 3 for Asian clams.

[f]$n = 6$.

Appendix 2. Recoveries of metals from National Institute of Standards reference materials.

[All concentrations in µg/g dry weight; ±, plus-or-minus]

Metal	Certified concentration	Measured concentration range	Recovery (percent)[a]
Standard Reference Material 1566a (oyster tissue, *n* = 3)			
Cobalt	0.57 ± 0.11	0.48–0.57	100
Nickel	2.25 ± 0.44	2.16–2.50	100
Zinc	830 ± 57	694–798	90–100
Cadmium	4.15 ± 0.38	4.04–4.32	100
Lead	0.371 ± 0.014	0.31–0.38	87–100
Standard Reference Material 1566b (oyster tissue, *n* = 4)			
Cobalt	0.371 ± 0.009	0.35–0.38	97–100
Nickel	1.04 ± 0.09	0.99–1.12	100
Zinc	1,424 ± 46	1,340–1,392	97–100
Cadmium	2.48 ± 0.08	2.43–2.51	100–101
Lead	0.308 ±0.09	0.29–0.31	97–100

[a]Recovery = 100 percent if within range, otherwise calculated based on upper or lower limit of reported concentration range.

Appendix 3. Carapace lengths of crayfish in composite samples from the indicated sites, and the coordinates of the approximate center of the location from which each sample was obtained.

[Carapace lengths in millimeters; OE, coldwater crayfish, *O. eupunctus*; OL, golden crayfish, *Orconectes luteus*; OP, spothanded crayfish, *O. punctimanus*; OH, belted crayfish, *O. harrison*i; CL1–Cl10, carapace lengths of individual crayfish; easting (X), northing (Y), and GPS accuracy (GPS Acc) in meters[a]; –, no data]

Sample	Species	CL1	CL2	CL3	Cl4	CL5	CL6	CL7	CL8	CL9	CL10	Mean	X	Y	GPS Acc
Site 1, Eleven Point River at Turner's Mill															
A	OE	25.3	22.8	20.6	22.5	23.7	21.6	24.1	22.9	21.7	19.1	22.4	654661	4070235	6.1
B	OE	22.2	19.3	21.0	19.8	21.2	22.8	21.5	21.9	23.4	19.3	21.2	654602	4070249	6.1
C	OE	21.6	23.7	23.5	21.3	22.3	21.7	23.4	25.0	17.1	18.9	21.9	654688	4070217	6.1
Site 2, Current River at Cataract Landing															
A	OL	24.3	21.8	20.4	19.9	23.1	17.4	18.0	17.4	12.5	13.8	18.9	686271	4085104	18.0
B	OL	26.4	25.1	22.9	20.3	18.8	18.3	17.8	15.9	–	–	20.7	686271	4085104	18.0
C	OL	27.8	22.3	23.4	20.1	20.4	18.0	17.4	17.2	17.2	18.2	20.2	686018	4084567	18.0
Site 3, Current River at Waymeyer Landing															
A	OL	23.3	17.9	18.1	17.7	17.7	18.0	16.9	17.6	15.9	15.5	17.9	673053	4102697	6.1
B	OL	25.3	23.9	18.3	18.4	18.7	17.8	17.8	17.4	17.2	17.0	19.2	673103	4102739	2.7
C	OL	18.6	24.4	21.2	18.8	18.8	17.5	19.1	17.1	17.6	17.0	19.0	673157	4102889	2.7
Site 4, Current River at Powdermill Ferry															
A	OL	20.1	24.4	24.0	17.8	20.1	19.0	17.9	18.6	19.1	20.0	20.1	661996	4116550	2.7
B	OL	20.3	20.4	25.3	22.5	24.5	23.6	21.0	20.7	23.2	22.8	22.4	661990	4116550	5.2
C	OL	23.7	21.8	20.0	20.1	20.0	21.6	19.5	21.9	17.1	17.7	20.3	662130	4116390	6.4
Site 5, Current River at Pulltite Landing															
A	OL	23.3	21.5	24.9	23.3	23.6	20.1	20.1	18.9	25.5	17.4	21.9	635022	4133046	5.8
B	OL	23.5	25.5	23.0	26.2	22.3	26.0	21.8	22.1	20.0	22.2	23.3	635046	4133034	4.9
C	OL	22.9	23.9	21.7	23.3	20.0	21.0	19.2	18.7	18.6	18.0	20.7	635107	4132969	5.5
Site 6, Jacks Fork at Shawnee Creek															
A	OL	22.9	22.4	22.3	19.9	21.2	18.9	16.1	15.7	15.3	15.5	19.0	650927	4115362	4.0
B	OL	22.8	21.6	22.4	17.0	18.4	16.4	17.0	16.7	17.0	17.0	18.6	650923	4115361	4.0
C	OL	22.2	23.8	26.7	21.2	20.4	17.2	17.5	15.4	17.4	16.8	19.9	650916	4115356	6.7
Site 7, Big River at St. Francois State Park															
A	OP	37.8	37.3	35.2	35.9	33.4	–	–	–	–	–	35.9	716069	4204186	5.5
B	OH	27.4	24.0	20.9	–	–	–	–	–	–	–	24.1	716069	4204186	5.8
C	OP	30.2	27.2	25.4	15.9	–	–	–	–	–	–	24.7	716069	4204186	5.8
D	OL	22.3	21.2	17.5	–	–	–	–	–	–	–	20.3	716069	4204186	5.8

[a] Universal Transverse Mercator coordinate system, NAD 83, Zone 15 N; coordinates obtained by Global Positioning System (GPS) receiver.

Appendix 4. Shell diameters of Asian clams in composite samples from the indicated sites, and the coordinates of the approximate center of the location from which each sample was obtained.

[Shell diameters in millimeters; D1–D10, diameters of individual Asian clams; easting (X), northing (Y), and GPS accuracy (GPS Acc)[a] in meters; –, no data]

Sample	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Mean	X	Y	GPS Acc
Site 1, Eleven Point River at Turner's Mill														
A	20.5	19.2	22.3	13.8	20.3	20.7	18.5	13.9	–	–	18.7	654692	4070235	7.0
B	22.7	21.1	19.9	20.9	20.3	17.4	20.7	–	–	–	20.4	654692	4070235	7.0
C	23.9	25.1	20.0	20.3	19.1	19.4	19.8	–	–	–	21.1	654692	4070235	7.0
Site 2, Current River at Cataract Landing														
A	22.5	18.4	19.8	18.2	16.0	16.9	17.7	15.4	13.8	15.0	17.4	685988	4084691	6.1
B	23.7	22.3	20.0	20.8	20.6	20.9	21.6	21.6	20.7	21.4	21.4	685952	4084586	6.1
C	22.1	21.4	20.9	22.2	19.3	20.2	21.5	21.1	21.3	19.6	21.0	686018	4084567	6.1
Site 3, Current River at Waymeyer Landing														
A	26.3	21.1	19.4	17.3	21.4	22.8	16.6	16.2	15.6	16.8	19.4	673014	4102580	5.2
B	24.0	19.3	17.8	17.7	16.4	18.2	17.0	17.1	18.4	16.9	18.3	673225	4102906	8.8
C	22.0	18.6	22.3	17.6	17.5	17.6	17.2	16.9	16.7	15.7	18.2	672936	4102584	2.4
Site 4, Current River at Powdermill Ferry														
A	22.7	16.5	15.7	16.4	16.4	15.5	15.9	16.4	16.5	14.8	16.7	662115	4116412	6.1
B	20.0	17.7	16.4	17.5	14.3	12.7	13.5	15.9	15.3	13.1	15.6	661775	4116722	5.8
C	17.9	18.5	15.5	13.8	13.7	14.9	13.2	16.5	14.8	16.4	15.5	661641	4117085	6.4
Site 5, Current River at Pulltite Landing														
A	22.8	17.5	19.7	17.4	17.9	18.5	18.2	17.6	16.4	16.8	18.3	635133	4132958	4.6
B	20.1	19.0	18.7	19.2	17.9	17.7	18.2	17.7	18.0	17.9	18.4	635162	4132940	7.6
C	17.7	17.5	15.0	17.0	16.5	16.4	17.2	15.4	16.6	16.3	16.6	635087	4133001	7.6
Site 6, Jacks Fork at Shawnee Creek														
A	17.4	16.0	15.5	17.5	20.4	17.0	16.6	16.1	16.5	14.2	16.7	650919	4115378	6.1
B	21.3	19.7	18.9	19.2	17.7	17.5	17.0	16.5	16.0	16.0	18.0	650929	4115375	4.9
C	17.7	18.6	18.0	17.5	17.1	16.5	16.2	15.9	16.2	14.9	16.9	650910	4115401	7.3
Site 7, Big River at St. Francois State Park														
A	14.7	12.2	12.6	12.3	12.6	14.0	13.3	14.9	13.8	13.7	13.4	716014	4204114	6.1
B	11.3	11.1	12.6	12.2	11.7	11.7	11.4	11.5	12.4	10.3	11.6	716014	4204114	6.1
C	12.6	9.7	9.4	10.6	9.8	10.3	11.1	10.5	12.7	12.0	10.9	716069	4204186	5.8

[a]Universal Transverse Mercator coordinate system, NAD 83, Zone 15 N; coordinates obtained by Global Positioning System (GPS) receiver.

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Schmitt and others—**Concentrations of Metals in Aquatic Invertebrates from the Ozark National Scenic Riverways, Missouri**—Open-File Report 2007–1435