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ABSTRACT

Crayfish (*Orconectes* spp.), Asian clam (*Corbicula fluminea*), northern hog sucker (hog sucker; *Hypentelium nigricans*), and smallmouth bass (smallmouth; *Micropterus dolomieu*) from streams in southeastern Missouri (USA) were analyzed for total mercury (HgT) and for stable isotopes of carbon (δ^{13}C), nitrogen (δ^{15}N), and sulfur (δ^{34}S) to discern Hg transfer pathways. HgT concentrations were generally lowest in crayfish (0.005–0.112 µg/g dw) and highest in smallmouth (0.093–4.041 µg/g dw), as was δ^{15}N. HgT was also lower and δ^{15}N was higher in all biota from a stream draining a more heavily populated historical lead–zinc mining area than from similar sites with mostly undeveloped forested watersheds. δ^{13}C in biota was lowest at spring-influenced sites, reflecting CO_2 inputs and temperature influences, and δ^{34}S increased from south to north in all taxa. However, HgT was not strongly correlated with either δ^{13}C or δ^{34}S in biota. Trophic position (TP) computed from crayfish δ^{15}N was lower in hog suckers (mean=2.8) than in smallmouth (mean=3.2), but not at all sites. HgT, δ^{13}C, δ^{34}S, and TP in hog suckers increased with total length (length) at some sites, indicating site-specific ontogenetic diet shifts. Changes with length were less evident in smallmouth. Length-adjusted HgT site means in both species were strongly correlated with HgT in crayfish ($r^2=0.97$, $P<0.01$), but not with HgT in *Corbicula* ($r^2=0.02$, $P>0.05$). ANCOVA and regression models incorporating only TP and, for hog suckers, length, accurately and precisely predicted HgT concentrations in both fish species from all locations. Although low compared to many areas of the USA, HgT (and therefore methylmercury) concentrations in smallmouth and hog suckers are sufficiently high to represent a threat to human health and wildlife. Our data indicate that in Ozark streams, Hg concentrations in crayfish are at least partly determined by their diet, with concentrations in hog suckers, smallmouth, and possibly other higher-level consumers largely determined by concentrations in crayfish and other primary and secondary consumers, fish growth rates, and TP.

1. Introduction

Mercury (Hg) is a ubiquitous pollutant released to the environment from natural and anthropogenic sources (US Environmental Protection Agency (USEPA), 2001). Much of the Hg in aquatic ecosystems originates from the atmosphere as inorganic Hg that reaches streams associated with leaf litter from the surrounding watershed (Wiener et al., 2006; Harris et al., 2007; Knightes et al., 2009). Biogeochemical processes convert inorganic Hg to methylmercury (MeHg), which is highly toxic, subject to bioaccumulation and biomagnification, and a potential threat to fish, wildlife, and human health (Neumann and Ward, 1999; Wiener et al., 2003; Hammerschmidt and Fitzgerald, 2006; Sandheinrich and Wiener, 2011). Most (>90 percent) of the Hg in fish and crayfish is MeHg (e.g., Wiener et al., 2003; Chasar et al., 2008). In the USA, nationwide human fish consumption guidance has been developed for Hg (as MeHg; US Environmental Protection Agency (USEPA),

2001), and most states have issued consumption advisories. Criteria vary, but in Missouri a statewide advisory is in effect for species with fillet concentrations that typically exceed 0.3 µg/g wet-weight (ww; Missouri Department of Health and Senior Services, MDHSS, 2010).

The southern Missouri Ozarks comprise dolomite and limestone karst. Many Ozark streams derive much of their flow from groundwater; streams, are fed by large conduit springs that are typically 13–15 °C year-round. Spring waters also contain higher concentrations of CO_2, dissolved organic carbon (DOC), and hardness ions, and have lower pH and dissolved oxygen concentrations than receiving streams, which they strongly influence (Vinyard and Feder, 1982; Imes et al., 2007; Mugel et al., 2009). Surface waters are hard, alkaline, and low in DOC, conditions not usually associated with Hg methylation or elevated MeHg concentrations in fish (e.g., Brumbaugh et al., 2001; Kamman et al., 2005; Brigham et al., 2009; Chumchall and Hambright, 2009). Nevertheless, concentrations of total mercury (HgT) in fillets of legal-size (>305 mm total length) smallmouth bass (*Micropterus dolomieu*; smallmouth) from Ozark streams typically exceed 0.3 µg/g ww (Schmitt and Brumbaugh, 2007) and are included

in the statewide consumption advisory (MDHSS, 2010). Mercury concentrations (as MeHg) tend to increase with age and body size in upper trophic level fishes (Wiener et al., 2003, 2006; McIntyre and Beauchamp, 2007), but size-concentration relations have not been evident in Ozark smallmouth (Schmitt and Brumbaugh, 2007). These relations can also be obscured by growth dilution (e.g., Ward et al., 2009), and growth rates can be influenced by the temperature stability afforded by springs (Whitledge et al., 2006). HgT concentrations also tend to be lower in smallmouth from streams that drain historical lead–zinc mining areas than elsewhere in southern Missouri (e.g., Schmitt and Brumbaugh, 2007).

Ozark stream ecosystems are supported by a mixture of autochthonous and allochthonous plant material that is processed by crayfish and other detritivores (e.g., Whitledge and Rabeni, 1997). The assimilation efficiency of leaf litter by aquatic invertebrates is low; the biofilm associated with conditioned detritus is of greater nutritional importance (Boling et al., 1975) and represents a site for Hg methylation (Desrosiers et al., 2006). Crayfish dominate the benthic macroinvertebrate community in Ozark streams and integrate the large, seasonal influx of terrestrial leaf litter (Rabeni, 1992; Whitledge and Rabeni, 1997). However, little is known about the transfer and bioaccumulation of Hg within these ecosystems. Our primary goal was to gain insight into the reasons for the Hg concentration differences among Ozark streams and the lack of size-concentration relations in smallmouth. We sought to expand on previous studies (Whitledge and Rabeni, 1997; Schmitt and Brumbaugh, 2007) by examining Hg dynamics in Ozark streams through a food web perspective. Our specific objectives were to (1) characterize HgT concentrations in representative primary and secondary consumer species (fish and invertebrates) from representative sites in Ozark streams; and (2) identify potential biological determinants (size, age, and trophic position) of Hg concentrations in fish.

2. Methods

We measured concentrations of HgT and the stable isotopes of carbon (C), nitrogen (N), and sulfur (S) in selected stream ecosystem components to gain insight into potential sources and pathways for Hg trophic transfer and biomagnification. The ratio of naturally occurring stable N isotopes (^{15}N:^{14}N; δ^{15}N) increases by 3–4 per mil (‰) per trophic level. Trophic position (TP), a determinant of Hg concentrations in aquatic organisms, can be inferred from δ^{15}N after normalizing to the base of the food web (Cabana et al., 1994; Cabana and Rasmussen, 1996; Vander Zanden et al., 1997; 2005; Vander Zanden and Rasmussen, 1999; Kendall, 1998; Lake et al., 2001; Franssen and Gido, 2006; Chasar et al., 2009; Payne and Taylor, 2010). Trophic discrimination is typically < 1‰ for stable C isotopes (^{13}C:^{12}C; δ^{13}C), but δ^{13}C can provide information about C sources such as C_3 vs. C_4 plants and algae vs. terrestrial detritus (Vander Zanden and Rasmussen, 1999; Finlay and Kendall, 2007; McCutchan et al., 2003). Methylation is typically facilitated by sulfate-reducing bacteria under reducing conditions and in the presence of organic C (Compeau and Bartha, 1985; Gilmour et al., 1992; Canavan et al., 2000; Schaefer and Morel, 2009), but may also involve iron reducers (Flanders et al., 2010). Some aquatic plants may also methylate Hg (Tsui et al., 2009). Sulfate may exert concentration-dependent positive or negative effects on Hg methylation and influence bioavailability (Scudder et al., 2009). Minimal S fractionation occurs during trophic transfer (McCutchan et al., 2003), but bacterial sulfate reduction increases ^{34}S:^{32}S (δ^{34}S) of residual SO_4 (Finlay and Kendall, 2007).

The samples we analyzed were also used in other studies. Additional information on the field and laboratory methods is available elsewhere (Schmitt and Brumbaugh, 2007; Schmitt et al., 2007, 2008, 2009).

2.1. Study area

Samples were collected in September–October, 2005 from sites on the Eleven Point River, Current River, Jacks Fork (JF), and Big River (BGR) affected to differing degrees by springs and nutrient sources (Fig. 1; Table 1). Two sites, the Eleven Point River at Turner's Mill (EPT) and the Current River at Cataract Landing (CCL), were directly downstream from major springs (Fig. 1). Greer Spring supplies > 50 percent of the base flow of the Eleven Point River at EPT (Imes et al., 2007). The Current and Eleven Point watersheds and the recharge areas of their large springs are sparsely populated, contain few pollution point-sources, and comprise mostly
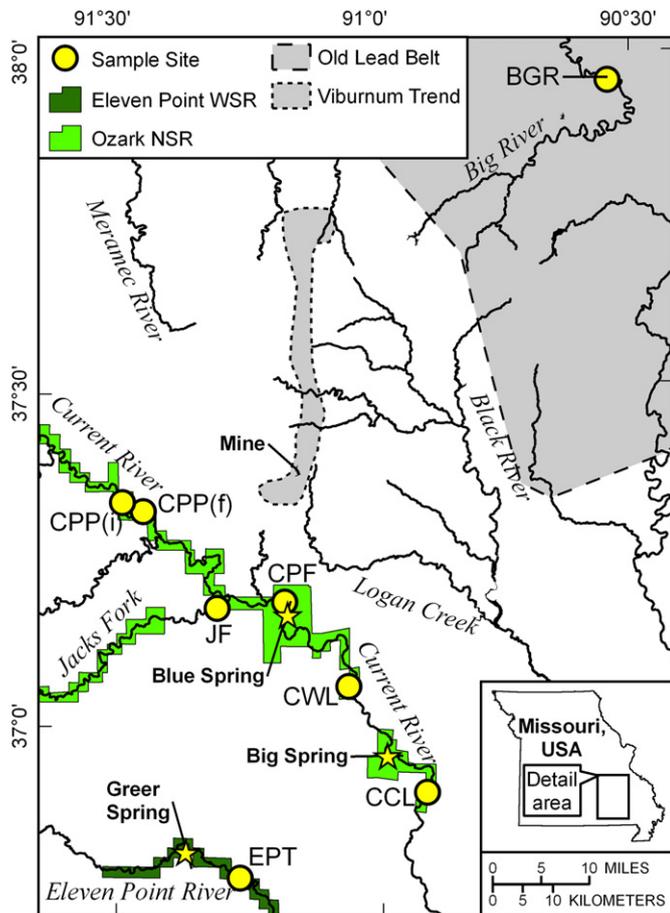
Fig. 1. Map showing collection sites, lead–zinc mining areas (Viburnum Trend, Old Lead Belt), counties, and boundaries of the Ozark National Scenic Riverways and the Eleven Point Wild and Scenic River. See Table 1 for locations.

Table 1
Collection sites for fish (f) and invertebrate (i; crayfish and *Corbicula*), and sample sources [a].

Site	River	Location	Latitude, longitude [b]	Source(s)
EPT[i, f]	Eleven Point	Turners Mill	36°45′56.7″N, 91°16′01.0″W	1, 2, 3
CPP[i]	Current	Pulltite Landing	37°20′04.1″N, 91°28′33.8″W	2
CPP[f]	Current	Presley Center	37°19′12.6″N, 91°26′14.6″W	1, 3
JF[i, f]	Jacks Fork	Shawnee Creek	37°10′21.3″N, 91°18′00.6″W	1, 2, 3
CPF[i, f]	Current	Powdermill Ferry	37°10′48.0″N, 91°10′25.0″W	2
CWL[i, f]	Current	Waymeyer Landing	37°03′15.1″N, 91°03′16.8″W	1, 2, 3
CCL[i, f]	Current	Cataract Landing	36°53′22.2″N, 90°54′473″W	1, 2, 3
BGR[i, f]	Big	St. Francois State Park	37°57′23.7″N, 90°32′29.2″W	1, 2, 3

[a] 1, Schmitt and Brumbaugh (2007); 2, Schmitt et al. (2007); 3, Schmitt et al. (2009).
[b] World Geodetic System (WGS) 84.

forest and pasture (Petersen et al., 1998; Miller and Wilkerson, 2000; Mugel et al., 2009). The upper Current River and most of the JF are within the Ozark National Scenic Riverways, a National Park; much of the Eleven Point River in Missouri is a federally designated Wild and Scenic River (Fig. 1). However, Blue Spring, on the Current River, is fed by a losing stream (Logan Creek) that receives drainage from an active lead–zinc mine in the Viburnum Trend mining district (Fig. 1; Schumacher, 2008), and there is nutrient enrichment and bacterial contamination at JF (Davis and Richards, 2001). There is less spring influence at BGR, and the watershed is more densely populated and less forested than those of the other rivers (Meneau, 1997). BGR is also contaminated by tailings from historical lead–zinc mining in the Old Lead Belt (Gale et al., 2004; Fig. 1). HgT concentrations in Missouri lead–zinc ores are comparatively low (Leach et al., 1995), but mine wastes represent significant sources of sulfides and sulfates to surface and groundwater (Brumbaugh et al., 2007; Kleeschulte, 2008; Schumacher, 2008) that could either facilitate Hg methylation or bind Hg and reduce its availability (Compeua and Bartha, 1985; Krabbenhoft et al., 1998; Marvin-DiPasquale et al., 2009). The BGR collection site was downstream of the Old Lead Belt, a population center, and a sewage treatment plant.

There are no known point sources of Hg pollution in southern Missouri. However, the rate of wet atmospheric deposition is comparatively high (8 – 18 µg/m²/yr), varying with rainfall (Prestbo and Gay, 2009). Dry deposition has not been measured, but the nearest atmospheric source is a coal-fired power plant > 125 km southeast of the study area. HgT concentrations in Ozark stream sediments are comparatively low (Petersen et al., 1998).

2.2. Species

Asian clam (Corbicula fluminea), crayfish (Orconectes spp.), northern hog sucker (Hypentelium nigricans; hog sucker), and smallmouth were selected as representative food web components of Ozark stream ecosystems. Corbicula is an introduced filter- and pedal-feeding bivalve chosen to represent the trophic pathway based on fine particulate organic matter (FPOM) originating from both allochthonous (terrestrial leaf litter) and autochthonous (in-stream algae) sources (McMahon, 1983). Crayfish in Ozark streams are omnivorous primary consumers that feed on coarse particulate organic matter derived mainly from leaf litter, but also consume varying proportions of periphyton, aquatic invertebrates, and fish (Rabeni, 1992; Hobbs, 1993; Whitledge and Rabeni, 1997; Stenroth et al., 2006). Crayfish also represent a significant food source for fish and riparian wildlife (Probst et al., 1984; Whitledge and Rabeni, 1997). Hog suckers are benthivorous omnivores that feed mainly on aquatic invertebrates and organic matter from the stream bottom (Pflieger, 1997; Rybczynski et al., 2009). In contrast to many other suckers (Catostomidae), hog suckers have a relatively small home range and do not migrate (Curry and Spacie, 1984; Matheny and Rabeni, 1995). Smallmouth in Ozark streams feed primarily on crayfish along with varying proportions of other benthic invertebrates and small fish (Probst et al., 1984). Ozark smallmouth also typically have restricted home ranges (Todd and Rabeni, 1989); however, they may move locally in response to temperature changes (Peterson and Rabeni, 1996), as do other fishes. Recreational fishers harvest and consume hog suckers and smallmouth from Ozark streams.

2.3. Field methods

At most sites, 12 fish of each species typically 200–500 mm total length (length), the range sought by recreational fishers, were collected by electrofishing. Fillet samples were removed from each fish in the manner typically employed by local sport fishers (skinless, boneless for smallmouth; skin-on, bone in for hog suckers). Scale samples were obtained for age determination. Three composite samples of 10 crayfish and Corbicula were obtained from each site. Juvenile crayfish (Orconectes spp.) of 12–18 mm carapace length, the size most often eaten by Ozark smallmouth (Probst et al., 1984), were collected by seining or dip-netting. Crayfish and fillet samples were frozen in the field with dry ice. Corbicula of 15–25 mm shell length were collected by hand, depurated for 24 h to allow them to purge ingested material, then frozen. All samples remained frozen (− 20 °C) until analysis.

2.4. Laboratory methods, quality assurance, and basic computations

Frozen composite samples of whole crayfish were lyophilized and cryogenically ground to ensure homogenization of the exoskeletons. Corbicula were thawed until the soft tissues could be separated from the shells, composited, lyophilized, and homogenized with a glass rod. Individual fillet samples were partly thawed, rinsed twice with ultrapure deionized water, and processed as described for Corbicula soft tissues.

All samples were analyzed for HgT by combustion-amalgamation atomic absorption spectrophotometry. Separate aliquots of hog sucker fillets were also analyzed for calcium (Ca) by inductively coupled plasma mass spectrometry as an indicator of the amount of bone, skin, and mucus included in each sample (Schmitt and Finger, 1987; Schmitt et al., 2009). QA measures included blanks, replicates,

instrument calibration standards, and certified reference materials analyzed with each group of samples. Method limits-of-detection were 0.001–0.010 µg/g dw for HgT and 3.2–11.0 mg/g for Ca, which were exceeded in all samples. Overall, the QA results indicated an acceptable level of precision and accuracy and no evidence of sample contamination (Supplemental Table S-1; Schmitt et al., 2009).

Additional aliquots of each sample were analyzed for stable isotopes. Bulk dried samples were analyzed for N and S. Sub-samples for C analysis were Soxhlet-distilled for 5 h in 2:1 chloroform:methanol followed by overnight drying at 25 °C prior to analysis to normalize for lipid-related $\delta^{13}C$ differences among samples and taxa (Post et al., 2007). Isotopes were analyzed by conventional continuous-flow isotope ratio mass spectrometry with an elemental analyzer coupled to a mass spectrometer (Fry et al., 1992). Results are reported in delta (δ) notation in parts-per-thousand (‰) deviation relative to a monitoring gas according to

$$\delta X = [(R_{sample}/R_{standard}) - 1],$$

where $X = {}^{15}N$, ${}^{13}C$, or ${}^{34}S$ and $R = {}^{15}N/{}^{14}N$, ${}^{13}C/{}^{12}C$ or ${}^{34}S/{}^{32}S$ in the sample and standard (monitoring gas). Isotope data were normalized using the following primary standards: USGS 40 and 41 [$\delta^{13}C = -26.24$ and -37.76‰ (respectively) relative to Vienna-Pee Dee Belemnite and $\delta^{15}N = -4.52$ and 47.57‰ relative to air]; and NBS127 and IAEA-SO-6 [$\delta^{34}S = 21.1$ and -34.05‰ (respectively) relative to Vienna-Canyon Diablo troilite]. Several in-house standards were also used as QA checks; analytical error and accuracy were generally < 0.2‰ and reproducibility of unknowns was typically ± 0.2‰ for $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$.

2.5. Statistical analyses

The Statistical Analysis System, Version 9.1 (SAS Institute, Cary, NC) was used for all analyses. Relations between pairs of variables within each taxon and site and across sites and taxa were examined initially by correlation analysis (Pearson) and simple linear regression. Analysis-of-variance (ANOVA) and analysis-of-covariance (ANCOVA) were used to test for differences among sites and to evaluate relations between and among variables; ANCOVA models evaluated regressions for both individual and pooled sites and species (Hebert and Keenleyside, 1995; Neumann and Ward, 1999; Barrett et al., 2010). Stable isotope ratios were considered both dependent variables and covariates. Length-adjusted HgT was computed and compared among sites and between fish species using ANCOVA. Fish and crayfish HgT were also evaluated using multiple linear regression with stepwise, forward, and Akaike's Information Criterion (adjusted for small sample sizes (AIC$_c$); Burnham and Anderson, 2002) selection methods. A value of $\alpha = 0.05$ was used to judge the significance of all statistical tests except stepwise and forward-selection multiple regression, for which the models containing the greatest number of independent variables that significantly ($P < 0.10$) reduced the unexplained sum-of-squares after accounting for all other variables in the model are reported; i.e., the Type-II and -III sums-of-squares were evaluated. In AIC regression, models with the lowest AIC$_c$ value were considered most parsimonious. The "best" regression models based on the forward selection, stepwise, and AIC analyses of HgT in fish and crayfish were nearly identical, so we report only the forward selection results. Fish weight, length, and HgT were log$_{10}$-transformed to meet the assumptions of parametric statistical methods prior to analysis. Although both arithmetic and geometric means and standard errors are presented and discussed, all statistical tests were based on transformed least-squares means, which are adjusted for all factors included in statistical models and are not biased by sample size.

A total of 202 Corbicula (21 composite samples) and 193 crayfish (22 samples) from all seven sites were collected and analyzed (Supplemental Table S-2). Crayfish and Corbicula size ranges were narrow (Supplemental Table S-2). Size was not correlated with other variables in either taxon and was eliminated from subsequent analyses. Hog suckers (total $n = 63$) and smallmouth ($n = 62$) were obtained from all sites except Current River at Powdermill Ferry (CPF), which was not sampled for fish. Male and female fish of both species were obtained everywhere except BGR, where only females and juveniles were collected. Fish of both species were smaller at JF and BGR than at the other sites (Supplemental Table S-3). Preliminary analysis of the fish data revealed that differences between genders were significant only for length in hog suckers (29 mm) and $\delta^{15}N$ (0.2‰) in smallmouth, both of which were considered inconsequential. Gender was therefore eliminated from subsequent analyses. Fish age spanned only 2–4 yr in both species at all sites; log$_{10}$-transformed length was therefore the most consistent representation of fish size and age and was used as such in most higher-level statistical analyses. Ca concentrations were significantly greater in hog suckers from EPT than elsewhere (Schmitt et al., 2009). Concentrations were also higher in the smaller hog suckers from JF, CPP, and BGR than in the larger fish from the other sites (Schmitt et al., 2009). Ca increased with fish size at CWL and CPP, but not at the other sites or when examined across all sites. Regression analysis indicated that Ca accounted for < 0.4 percent of the total variation in hog sucker HgT and it was excluded from subsequent analyses. These results also indicated that the amount of tissue other than muscle included in the fillets did not strongly influence HgT concentrations in hog sucker fillets. $\delta^{15}N$ was substantially higher at BGR than elsewhere (Supplemental Tables S-2, S3), so only

TP was used in analyses of fish data that involved multiple sites. BGR also differed in other respects from the other sites and was excluded from some analyses.

The small *Corbicula* from BGR provided insufficient mass for analysis of stable isotopes; however, crayfish and *Corbicula* $\delta^{15}N$ were similar at the six sites with data for both taxa (Supplemental Table S-2). We therefore computed the TP of hog suckers and smallmouth relative to crayfish as

$$TP = [(\delta^{15}N_{fish} - \delta^{15}N_{crayfish})/3.4] + 2.0,$$

where $\delta^{15}N_{fish}$ is the $\delta^{15}N$ value of an individual fish, $\delta^{15}N_{crayfish}$ is the corresponding site mean for crayfish, 3.4 is a widely accepted trophic discrimination factor, and 2.0 represents a basal consumer level (Franssen and Gido, 2006).

Biomagnification factors (BMFs) for Hg were calculated as

$$BMF = HgT_{fish}/HgT_{food},$$

where HgT_{fish} = consumer (smallmouth, hog sucker) concentration and HgT_{food} = food (*Corbicula*, crayfish, hog sucker) concentration (Herrin et al., 1998; DeForest et al., 2007).

2.6. Ethics statement

All field procedures conformed to Scientific Investigator and Wildlife Collector permits issued by the US National Park Service (NPS) and the Missouri Department of Conservation (MDC), respectively; with recommendations of the American Fisheries Society (AFS), American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists (AFS et al., 2004); and with requirements of the US Laboratory Animal Welfare Act, the Interagency Research Animal Committee, and all USGS guidelines for the humane treatment of test organisms during culture and experimentation.

3. Results

3.1. HgT

At sites where both fish and invertebrates were collected (Table 1), HgT was uniformly lowest in whole crayfish and highest in smallmouth fillets (Fig. 2; Supplemental Tables S-2, S-3). Differences among sites were significant in all taxa except *Corbicula*. Concentrations were lowest in all taxa at BGR, although not significantly so in *Corbicula*, and highest in crayfish, hog suckers, and smallmouth at CPP (Fig. 2; Supplemental Tables S-2, S-3). Crayfish HgT was also comparatively high at CPF, which is near CPP but where fish were not collected (Fig. 2). Concentrations at EPT, which is directly downstream from Greer Spring, were comparatively low in all taxa (Fig. 2). In *Corbicula* soft tissues, HgT was uniformly higher than in crayfish by an average
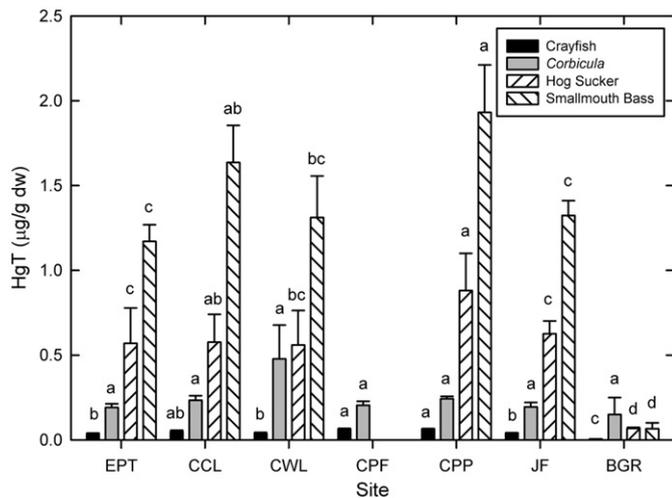
of eight-fold, but *Corbicula* site means differed by only about 3-fold (Fig. 2; Supplemental Table S-2).

Across all sites, hog sucker HgT averaged seventeen-fold higher than in crayfish and three-fold higher than in *Corbicula* (Fig. 2; Supplemental Tables S-2, S-3). Hog sucker HgT increased significantly with length ($r = 0.79 - 0.92$, $P < 0.01$) at all sites except BGR (EPT not evaluated; $n = 3$) and also across all sites (Fig. 3; Table 2). Site alone accounted for 82 percent of the variation in hog sucker HgT among sites, with length accounting for an additional 10–11 percent Supplemental Table S-4. Without BGR, site and length together accounted for 67 percent. Among-site differences in hog sucker HgT remained evident (and significant) after accounting for fish size, but the differences were smaller (Fig. 4; Supplemental Table S-3). Relative to crayfish, the BMF for HgT in hog suckers was lower at BGR (~ 10) than elsewhere (14 at CPP to 22 at CWL). Hog sucker BMFs were more uniform after adjusting for fish size: 9.9–11.6 at BGR, JF, and CCL, and 14.0–14.9 at EPT, CWL, and CPP.
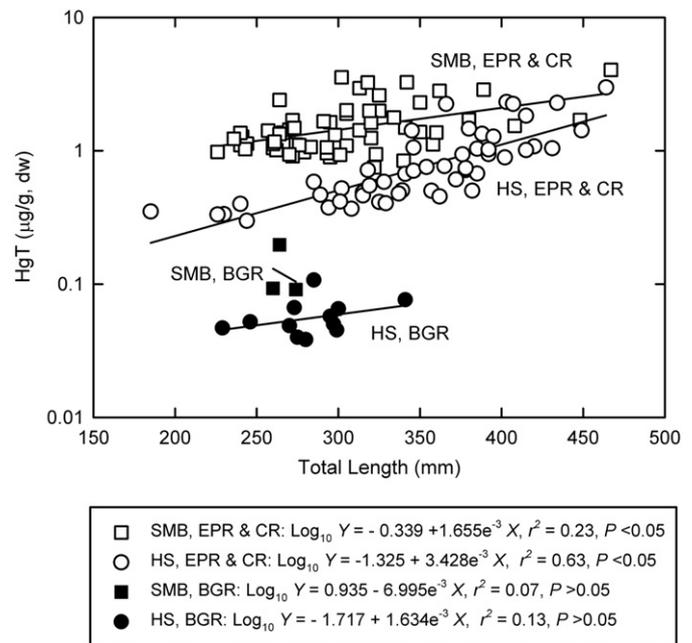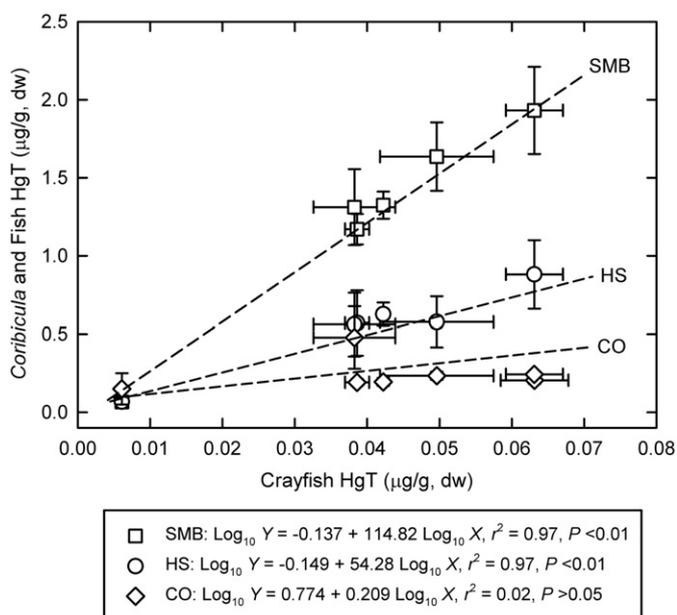
Fig. 3. Total mercury (HgT; Y) vs. total length (X) in hog suckers (HS) and smallmouth bass (SMB) from the Current River (CR), Eleven Point River (EPR), and Big River (BGR).

Legend:
- □ SMB, EPR & CR: $Log_{10} Y = -0.339 + 1.655e^{-3} X$, $r^2 = 0.23$, $P < 0.05$
- ○ HS, EPR & CR: $Log_{10} Y = -1.325 + 3.428e^{-3} X$, $r^2 = 0.63$, $P < 0.05$
- ■ SMB, BGR: $Log_{10} Y = 0.935 - 6.995e^{-3} X$, $r^2 = 0.07$, $P > 0.05$
- ● HS, BGR: $Log_{10} Y = -1.717 + 1.634e^{-3} X$, $r^2 = 0.13$, $P > 0.05$

Table 2
Pearson correlation coefficients (*$P < 0.05$; **$P < 0.01$) for relations between the indicated variables in smallmouth bass (above the principal diagonal) and hog suckers (below the principal diagonal)[a].

Hog sucker	Smallmouth bass							
	Length	Weight	Age	TP	$\delta^{15}N$	HgT	$\delta^{13}C$	$\delta^{34}S$
Length	–	0.98**	0.75**	0.31**	0.12	0.48**	−0.29**	−0.07
Weight	0.99**	–	0.70**	0.35**	0.15	0.48**	−0.27*	−0.17
Age	0.72**	0.76**	–	0.22	0.05	0.54**	−0.19	−0.12
TP	0.46*	0.46*	0.57**	–	0.57**	0.08	−0.41**	−0.61**
$\delta^{15}N$	0.39**	0.46**	0.32*	0.60**	–	−0.13	0.19	−0.37**
HgT	0.79**	0.79**	0.79**	0.79**	0.41**	–	−0.24	0.27*
$\delta^{13}C$	−0.02	0.13	< 0.01	−0.14	0.44**	0.04	–	−0.15
$\delta^{34}S$	0.26	0.22	0.09	0.36**	0.45**	0.20	−0.40**	–

[a] All sites for length, weight, age, and total mercury (HgT; $n = 63$ for hog suckers, 62 for smallmouth bass); all sites except BGR for trophic position (TP), $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ ($n = 51$ for hog suckers, 59 for smallmouth bass).

Fig. 2. Total mercury (HgT) arithmetic site means ($\pm$ standard errors) in *Corbicula*, crayfish, hog suckers, and smallmouth bass. Within taxa, means sharing the same letter are not significantly different (ANOVA, $P < 0.05$). See Fig. 1 and Table 1 for site names and locations.

SMB: $Log_{10} Y = -0.137 + 114.82 Log_{10} X$, $r^2 = 0.97$, $P < 0.01$

HS: $Log_{10} Y = -0.149 + 54.28 Log_{10} X$, $r^2 = 0.97$, $P < 0.01$

CO: $Log_{10} Y = 0.774 + 0.209 Log_{10} X$, $r^2 = 0.02$, $P > 0.05$

Fig. 4. Total mercury (HgT) in *Corbicula* (CO) soft tissues and in fillets of hog sucker (HS) and smallmouth bass (SMB; all Y) vs. HgT in whole crayfish (X). Shown are geometric site means ($\pm$ standard errors), with concentrations in fish adjusted to the grand mean total length of each species (HS=337 mm, SM=318 mm) via ANCOVA using species and site-specific linear regressions.

Smallmouth HgT increased with length at only two sites, CWL and CPP ($r=0.60-0.65$, $P < 0.05$; Fig. 3). Across all sites, smallmouth HgT was more strongly correlated with fish age than with length or weight than in hog suckers (Table 2), but not at individual sites. Site alone accounted for 72 percent of the total variation in smallmouth HgT among sites, with length accounting for an additional 7–8 percent (Supplemental Table S-4). Without BGR, site and length together accounted for only 42 percent. Relative differences among sites were similar regardless of whether fish size was incorporated into the analysis except at BGR, where length-normalized HgT in hog suckers and smallmouth were nearly identical (Fig. 4; Supplemental Table, S-3). Smallmouth HgT remained correlated with size and age across all sites when BGR was excluded, however. Across all sites, mean BMFs for smallmouth fillets were 32 relative to whole crayfish, 4.0 relative to *Corbicula* soft tissues, and 2.0 relative to hog sucker fillets. Smallmouth BMFs relative to hog suckers were more consistent (1.7–2.2) than BMFs relative to crayfish. Relative to crayfish the BMF was 21 at BGR, substantially lower than all other sites (30 at CPP to 39 at CCL) and similar to the BMF for hog suckers. Smallmouth BMFs based on length-adjusted HgT were also substantially lower relative to crayfish at BGR (11) than elsewhere (21 at JF, 30–34 at all other sites).

Mean HgT in hog suckers and smallmouth was strongly correlated with mean crayfish HgT when examined across sites. Linear regressions between crayfish and fish site means explained > 97 percent of the variation in length-adjusted fillet HgT in both fish species (Fig. 4). In contrast, and despite the substantially higher concentrations in *Corbicula* than in crayfish, mean *Corbicula* HgT was not correlated with HgT in the other three taxa (Fig. 4).
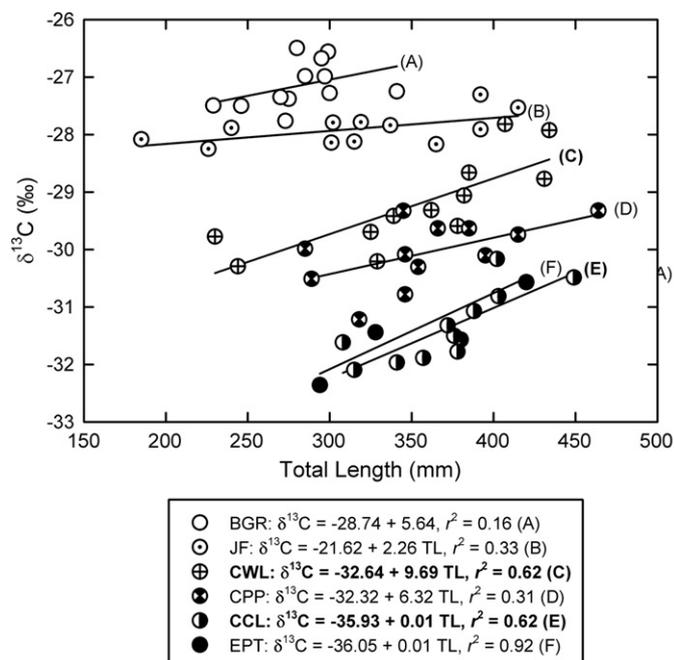
3.2. Stable isotopes and TP

Mean TP in hog suckers (relative to crayfish) differed significantly among sites, averaging 3.1 at CPP but only 2.6–2.8 elsewhere. Hog sucker TP increased significantly with length at CWL, CPP, and JF, but not at the other sites or across all sites (Table 2;

Supplemental Table S-3). The overall mean TP for hog suckers was 2.8. Smallmouth TP also differed significantly among sites and was typically higher than in hog suckers, but not at all sites. Smallmouth TP site means ranged from 3.1 at CWL, CPP, and JFR to 3.5 at EPT, with an overall mean of 3.2 (Supplemental Table S-3). Differences between smallmouth and hog sucker site means ranged from 0.0 at CPP to 0.8 at EPT (Supplemental Table S-2). Smallmouth TP increased with length across all sites with BGR excluded (Table 2) and at EPT ($r=0.61$, $P < 0.05$), but not at any other individual sites.

Spring influence was indirectly expressed in the $\delta^{13}C$ results; $\delta^{13}C$ was significantly lower (by about 2‰) in most taxa at the two sites located directly downstream of large springs (EPT and CCL) than elsewhere (Supplemental Table S-3). Conversely, the highest $\delta^{13}C$ values in crayfish, hog suckers, and smallmouth were at BGR (*Corbicula* from BGR were not analyzed for stable isotopes) and JF, the sites least influenced by springs. At EPT and CCL, the lowest values were in *Corbicula* and hog suckers (Supplemental Tables S-2, S-3). Differences among taxa were smaller at the other sites. In hog suckers, $\delta^{13}C$ increased significantly with fish size at CCL, CWL, and JF, but not at the other sites or across all sites (Fig. 5; Table 2). In smallmouth, $\delta^{13}C$ increased significantly with fish size only at EPT and JF ($r=0.65-0.71$, $P < 0.05$; Table 2). In hog suckers, $\delta^{13}C$ and TP were positively correlated ($r=0.66-0.99$, $P < 0.05$) at all sites except CCL and BGR; at BGR the correlation was negative ($r=-0.66$, $P < 0.05$). $\delta^{13}C$ and TP were not significantly correlated in smallmouth at any sites or across all sites in either species (Table 2), but were negatively correlated in smallmouth across sites with BGR excluded (Table 2).

$\delta^{34}S$ was typically highest in crayfish (mean=5.0‰) followed by smallmouth (3.3‰), hog suckers (3.2‰), and *Corbicula* (2.4‰; no *Corbicula* data for BGR; Supplemental Tables S-2, S-3). Values for hog suckers were lower than for smallmouth at most sites, but at EPT and BGR they were similar (Supplemental Tables S-2, S-3). Mean $\delta^{34}S$ also differed significantly among sites in all taxa. A general north-south trend was evident, with $\delta^{34}S$ generally highest at sites in the upper Current River watershed and at BGR and

BGR: $\delta^{13}C = -28.74 + 5.64$, $r^2 = 0.16$ (A)

JF: $\delta^{13}C = -21.62 + 2.26$ TL, $r^2 = 0.33$ (B)

CWL: $\delta^{13}C = -32.64 + 9.69$ TL, $r^2 = 0.62$ (C)

CPP: $\delta^{13}C = -32.32 + 6.32$ TL, $r^2 = 0.31$ (D)

CCL: $\delta^{13}C = -35.93 + 0.01$ TL, $r^2 = 0.62$ (E)

EPT: $\delta^{13}C = -36.05 + 0.01$ TL, $r^2 = 0.92$ (F)

Fig. 5. $\delta^{13}C$ (Y) vs. total length (X) of hog suckers from the study sites. Regression results shown in bold are statistically significant ($P < 0.05$). See Fig. 1 and Table 1 for site names and locations.

lowest at EPT (Supplemental Tables S-2, S-3). Across sites, $\delta^{34}S$ was negatively correlated with TP and $\delta^{15}N$ in smallmouth, but increased with both in hog suckers (Table 2) with or without data from BGR.

3.3. HgT relative to stable isotopes and TP

Crayfish HgT was negatively correlated with $\delta^{15}N$ across the six sites with complete data ($r = -0.95$, $P < 0.01$), but not with BGR excluded ($r = -0.11$, $P > 0.05$). Crayfish HgT was not correlated with $\delta^{13}C$ with or without data for BGR. A statistically significant multiple linear regression model containing a negative coefficient for $\delta^{15}N$ and a positive coefficient for $\delta^{34}S$ explained 92 percent of the variation in HgT for all crayfish samples, with 90 percent accounted for by $\delta^{15}N$ (Supplemental Table S-4). No models for HgT in crayfish were significant with BGR excluded, nor was HgT in *Corbicula* significantly correlated with any other variables.

With BGR excluded, hog sucker HgT increased with TP and $\delta^{34}S$ across the remaining sites (Fig. 6; Table 2) and at CWL and CPP ($r = 0.82 - 0.91$, $P < 0.01$), but not at any other individual sites. Hog sucker HgT also increased with $\delta^{13}C$ at CCL, CWL, CPP, and JFR ($r = 0.59 - 0.92$, $P < 0.05$), but not at BGR ($r = -0.18$, $P > 0.05$) or across all sites (Table 2). ANCOVA indicated that across all sites, pooled and site-specific TP and $\delta^{13}C$ were statistically significant but explained only an additional 2–3 percent of the total variation in HgT beyond what was accounted for by only site and length (Supplemental Table S-4). Without BGR, TP and $\delta^{13}C$ accounted for an additional 5–13 percent. A multiple regression model containing a positive coefficient for pooled length and negative coefficients for pooled $\delta^{13}C$ and $\delta^{34}S$ explained 62 percent of the variation in hog sucker HgT across all sites. Without BGR, a model that also included a positive term for pooled TP but not $\delta^{34}S$ explained 72 percent (Supplemental Table S-4).

Smallmouth HgT was significantly correlated only with fish size and $\delta^{34}S$ across sites (Table 2) and with TP only at individual sites and not across sites (Fig. 5). Consequently, ANCOVA indicated that site-specific TP explained only an additional 6 percent

of the variation in HgT beyond that explained by site and length across all sites without BGR. Pooled TP was not significant in smallmouth (Supplemental Table S-4). Across all sites, a multiple regression model containing positive terms for length and TP and a negative coefficient for $\delta^{13}C$ explained 42 percent of the variation in smallmouth HgT. Without BGR, a model containing only $\delta^{13}C$ and $\delta^{34}S$ explained 32 percent (Supplemental Table S-4).

ANCOVA models fit to the combined smallmouth and hog sucker data set were similar to those for each species considered separately. Models containing positive coefficients for site-specific length, TP, $\delta^{13}C$, and $\delta^{34}S$ were statistically significant ($P < 0.01$) and accounted for up to 94 percent of the variation in HgT across all sites. Without BGR, the combined-species models explained up to 90 percent of the variation in HgT (Supplemental Table S-4). Multiple regression models fit to the pooled data explained 44 percent of the total variation in HgT across all sites and 59 percent without BGR (Supplemental Table S-4). Collectively, the ANCOVA, regression, and correlation results indicate that most of the among-site variation in both species was due to differing Hg concentrations in lower trophic levels (here represented by crayfish), fish size, and TP.

4. Discussion

4.1. Mercury bioaccumulation by crayfish and Corbicula

Concentrations of HgT in whole crayfish from the Current and Eleven Point Rivers ($0.030 - 0.071 \, \mu g/g \, dw$) were within the range of the lowest concentrations reported for whole crayfish from uncontaminated sites in other areas (Rada et al., 1986; Mason et al., 2000; Castro et al., 2007; Larsson et al., 2007; McIntyre and Beauchamp, 2007; Suárez-Serrano et al., 2010). Concentrations were substantially lower (mean$=0.006 \, \mu g/g \, dw$) at BGR. In contrast, much higher ($>$ ten-fold) concentrations have been documented, especially at Hg-contaminated sites (Hoffman and Curnow, 1979; Scheuhammer and Graham 1999; Hildebrand et al., 1980; Chasar et al., 2008; Suchanek et al., 2008b; Suárez-Serrano et al., 2010). Although the concentrations were comparatively low, our site means differed by fifteen-fold (3.6 without BGR).

Crayfish efficiently assimilate and retain MeHg from food (Headon et al., 1996; Simon et al., 2000; Lake et al., 2007). Consequently, MeHg as a percentage of HgT in whole crayfish is typically >80 percent (Haines et al., 2003; Chasar et al., 2008). The range of concentrations represented by our data and previous reports in the scientific literature, together with the correlation between HgT in crayfish and other taxa, no doubt reflect the close coupling of fish and crayfish in Ozark streams (e.g., Rabeni, 1992) and indicate that crayfish are important in the trophic transfer of Hg.

Extrapolation of previously published $\delta^{13}C$ and $\delta^{15}N$ data for the upper JF (Whitledge and Rabeni, 1997) to our HgT and stable isotope data provides evidence that bioaccumulation by crayfish is at least partly determined by crayfish diet. Mean $\delta^{13}C$ was 0.7–2.6‰ higher in crayfish than in *Corbicula* at all sites where both taxa were analyzed (i.e., all except BGR; Supplemental Tables S-2, S-3), which indicates differing C sources for crayfish and *Corbicula*. However, and in contrast to $\delta^{13}C$, our crayfish and *Corbicula* $\delta^{15}N$ site means differed by only -0.4 to 0.8‰. Because algal and detrital $\delta^{15}N$ in the upper JF differed substantially, the crayfish $\delta^{15}N$ data imply similar N sources and appear to contradict the $\delta^{13}C$ data. Further extrapolation of upper JF $\delta^{15}N$ to our data offers an alternative explanation, which is the consumption of animal material (invertebrates and fish) by crayfish. The incorporation of other invertebrates ($\delta^{15}N$ approximately 5‰) into crayfish diets

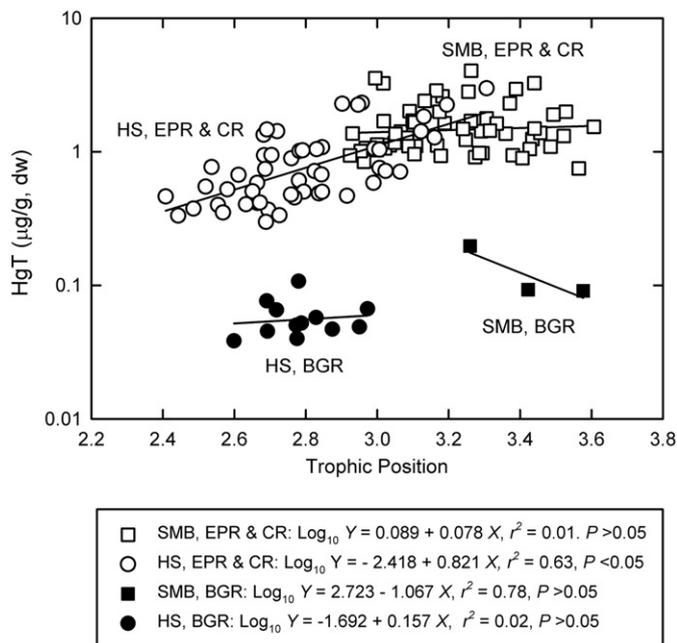**Fig. 6.** Total mercury (HgT; *Y*) vs. trophic position (TP; *X*) in hog suckers (HS) and smallmouth bass (SMB) from the Current River (CR), Eleven Point River (EPR), and Big River (BGR).

Legend within figure:
- □ SMB, EPR & CR: $Log_{10} Y = 0.089 + 0.078 X$, $r^2 = 0.01$. $P > 0.05$
- ○ HS, EPR & CR: $Log_{10} Y = -2.418 + 0.821 X$, $r^2 = 0.63$, $P < 0.05$
- ■ SMB, BGR: $Log_{10} Y = 2.723 - 1.067 X$, $r^2 = 0.78$, $P > 0.05$
- ● HS, BGR: $Log_{10} Y = -1.692 + 0.157 X$, $r^2 = 0.02$, $P > 0.05$

would increase $\delta^{15}N$ substantially over a diet based exclusively on stream-conditioned leaf litter ($\delta^{15}N$ approximately -1.5 to 1‰). In the upper JF, 30–50 percent of crayfish production is derived from invertebrate consumption (Whitledge and Rabeni, 1997). Juvenile crayfish also molt frequently and eat their exoskeleton, which contains chitin and is enriched in $\delta^{15}N$ relative to other tissues (Wang et al., 2007), and crayfish may eat fish or be cannibalistic (Capelli, 1980; Knowlton et al., 1983; Whitledge and Rabeni, 1997). Growth rate differences resulting in more frequent molts would tend to increase $\delta^{15}N$ but not HgT, whereas carnivory (including cannibalism) would increase both. Overall, the amount of animal material in the crayfish diet appears to be an important determinant of their Hg concentration, which has been demonstrated experimentally (Bowling et al., 2011). As noted by Pennuto et al. (2005), the influence of invertebrate feeding habits on Hg dynamics in aquatic ecosystems has been greatly underappreciated.

In contrast to crayfish, HgT concentrations in Corbicula were similar across our sites and were not correlated with concentrations in other taxa. This finding may reflect the de-coupling of Corbicula, but not necessarily other invertebrate FPOM consumers, from the aquatic food web. In addition (or perhaps alternatively), extant evidence indicates that Corbicula can demethylate and excrete MeHg more rapidly than other taxa, and thereby at least partly regulate HgT concentrations (Simon and Boudou, 2001; Foe et al., 2003). The latter explanation also implies that HgT concentrations in Corbicula may not reflect environmental concentrations or exposure as accurately as concentrations in crayfish.

4.2. TP and mercury biomagnification in hog suckers and smallmouth

Suckers are not widely pursued for human consumption. Consequently, fewer studies have documented Hg bioaccumulation in fillets of suckers (Catostomidae) than in smallmouth and other more popular game species, and still fewer have examined relations with fish size or TP. Relative to previous studies, concentrations in Ozark hog suckers (0.298–2.988 μg/g dw) were generally similar to those reported in suckers from uncontaminated sites elsewhere (e.g., Christensen et al., 2006; Kamman et al. 2005). Concentration changes with fish size in catostomids have been reported, but vary in direction and magnitude. For example, HgT increased with fish size in white sucker (Catostomus commersonii) and hog sucker fillets from several areas in central and northeastern North America (Christensen et al., 2006; Kamman et al., 2005) whereas both $\delta^{15}N$ and HgT decreased with fish size and age in white suckers from six lakes in eastern Canada (Kidd et al., 1995). Kidd et al. (1995) attributed the decreases to ontogenetic diet shifts and basal $\delta^{15}N$ differences among the lakes due to factors such as nutrient availability, epilimnetic temperatures, and species composition. Inconsistent HgT increases with fish weight in hog suckers from an Hg-contaminated site in Virginia were also attributed to among-site growth rate differences (Hildebrand et al., 1980). Our finding of $\delta^{15}N$, $\delta^{13}C$, and HgT changing with length in hog suckers at some sites indicates site-specific ontogenetic diet shifts. Although not considered a sport fish in most of the USA, large numbers of suckers (including hog suckers) are captured and eaten by Ozark recreational fishers. Our data indicate that fillets of hog suckers >350 mm (total length) may contain as much HgT (and therefore MeHg) as similar-sized smallmouth and could therefore represent the same risk. Suckers are also important in the diets of some riparian wildlife species (e.g., Johnson et al., 2008), which might also be at risk (Scheuhammer and Graham, 1999).

Although HgT (and therefore MeHg) concentrations in Ozark smallmouth fillets are sufficiently high to warrant restricted human consumption (0.091–4.041 μg/g dw; MDHSS, 2010), they are also comparable to or lower (at BGR) than concentrations in stream-dwelling smallmouth from streams in other areas of the US and Canada that have not been directly influenced by Hg from mining or industrial sources (e.g., Brumbaugh et al., 2001; Kamman et al., 2005; Peterson et al., 2005). Concentrations are also lower than those typical of lake-dwelling smallmouth, smallmouth from contaminated sites, and more strictly piscivorous freshwater species such as largemouth bass (Micropterus salmoides), walleye (Sander vitreum), or northern pike (Esox lucius; e.g., Hildebrand et al., 1980; Brumbaugh et al., 2001; US Environmental Protection Agency (USEPA), 2001; Neumann and Ward, 1999; Lake et al., 2007; Christensen et al., 2006).

5. Conclusions

Concentrations of HgT in fish from Ozark streams are low compared to many other areas in North America, but are nevertheless sufficiently high to warrant restricted human consumption and to represent a risk to wildlife. Although HgT concentrations in crayfish are also low, their prevalence in the benthic fauna and dietary importance to smallmouth and other higher-level consumers seems to ensure MeHg transfer. Direct consumption of juvenile crayfish, aquatic insect larvae, and detritus is consistent with the feeding ecology and TP of hog suckers. Similarly, consumption of crayfish, other invertebrates, and fish by smallmouth is consistent with their generally higher HgT concentrations and TP. There was little change in any of the variables we measured with smallmouth length or age, indicating a relatively narrow niche breadth. Conversely, the increasing HgT concentrations and stable isotope changes with size in hog suckers at some sites indicate a wider niche breadth and site-specific ontogenetic diet shifts. Incorporation of invertebrates and fish into crayfish diets, which would increase Hg biomagnification over what would be expected from consumption of stream-conditioned detritus alone, represents a plausible explanation for among-site differences in crayfish HgT. At BGR, we suspect that low HgT concentrations in biota reflect the combined effects of FPOM dilution by mine tailings and other inorganic sediments from construction and agriculture, biodilution by algal blooms (e.g., Chen and Folt, 2005), reduced flux of terrestrial detritus from the more heavily developed watershed, and lower bioavailability and possibly methylation rates due to sulfides in the lead–zinc mine wastes (e.g., Suchanek et al., 2008a; Marvin-DiPasquale et al., 2009). The comparative uniformity of the HgT accumulation patterns among taxa and sites also indicates that once incorporated into the food web, differences among sites (including BGR) in the proportion of HgT represented by MeHg are small. The high degree of correlation between HgT in juvenile crayfish and both species of fish at all of our sites also indicates a uniformly high MeHg percentage in crayfish, as has been reported elsewhere (Haines et al., 2003; Pennuto et al. 2005; Chasar et al. 2008).

In contrast to crayfish, HgT concentrations in Corbicula were not correlated with concentrations in any of the other taxa we analyzed, which further reinforces the apparent importance of crayfish and possibly other consumers of conditioned detritus in the trophic transfer of MeHg. The lack of correlation may also reflect the de-coupling of Corbicula from the ecosystem, MeHg excretion by Corbicula (Simon and Boudou, 2001; Foe et al., 2003), or both. It also does not rule out the potential importance of other FPOM-dependent invertebrates as vectors for MeHg accumulation by higher consumers. In addition, and despite sewage inputs and the presence of springs, $\delta^{13}C$ spanned a relatively narrow range

(< 6‰) compared to other ecosystems. And although $\delta^{13}C$ spatial patterns related to springs were clearly evident, HgT concentrations were not strongly correlated with $\delta^{13}C$. These findings, together with comparatively low HgT concentrations in all taxa at the site with the most spring influence (EPT), indicate that although springs profoundly influence water quality and may represent a C source to aquatic primary producers, springs do not appear to represent Hg sources. Data from the spring branches themselves would be required to ascertain their Hg contributions, however. The mining-derived sulfates originating from Blue Spring may also affect methylation rates, as may stream temperature. The latter may also influence Hg concentrations in fish through effects on feeding and growth rates (e.g., Norstrom et al., 1976; Whitledge et al., 2006; Ward et al., 2009).

Our data indicate only subtle differences in trophic structure among Ozark streams. We suspect that HgT concentrations in smallmouth and other fishes reflect differences in Hg concentrations in crayfish, which are determined by Hg transport from the catchments, Hg bioavailability, and in-situ methylation rates along with the TP, growth rate, and age of the crayfish and other consumer organisms, including fish. The similar HgT concentrations in smallmouth and large hog suckers at many sites is consistent with their similar TPs. Worthwhile topics for future research include determining whether temporal variation in rainfall and Hg influx is reflected in crayfish HgT; documenting spatial variation in methylation rates and MeHg concentrations in water, crayfish, and other biota (including autotrophs) as they might be influenced by sulfides, sulfates, other factors associated with lead–zinc mining, hydrology, and watershed characteristics; investigation of spatial and temporal variation in crayfish trophic dynamics; further documentation of Hg demethylation and excretion by *Corbicula*; and determining temperature influences on methylation rates, fish growth, and MeHg bioaccumulation relative to springs.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2011.08.008.

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