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Influence of relative trophic position and carbon source on selenium bioaccumulation in turtles from a coal fly-ash spill site

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ABSTRACT

Selenium (Se) is a bioaccumulative constituent of coal fly-ash that can disrupt reproduction of oviparous wildlife. In food webs, the greatest enrichment of Se occurs at the lowest trophic levels, making it readily bioavailable to higher consumers. However, subsequent enrichment at higher trophic levels is less pronounced, leading to mixed tendencies for Se to biomagnify. We used stable isotopes (^{15}N and ^{13}C) in claws to infer relative trophic positions and relative carbon sources, respectively, of seven turtle species near the site of a recently-remediated coal fly-ash spill. We then tested whether Se concentrations differed with relative trophic position or relative carbon source. We did not observe a strong relationship between $\delta^{15}N$ and Se concentration. Instead, selenium concentrations decreased with increasing $\delta^{13}C$ among species. Therefore, in an assemblage of closely-related aquatic vertebrates, relative carbon source was a better predictor of Se bioaccumulation than was relative trophic position.

1. Introduction

Coal fly-ash contains elevated concentrations of many trace elements that can pose health risks to local plants, wildlife, and humans (Rowe et al., 2002). Selenium (Se) is a primary driver of ecological risk in aquatic systems impacted by coal fly-ash (Cherry and Guthrie, 1977; Hopkins et al., 2002, Rowe et al., 2002; Young et al., 2010). Unlike most other bioaccumulative contaminants, Se is an essential trace element in vertebrates at low concentrations, but becomes toxic at higher concentrations (Janz et al., 2010; Lemly, 1995; Tinggi, 2003). Toxicity may arise through multiple biochemical pathways (Janz et al., 2010), but most often manifests as reproductive impairment and/or teratogenicity (Janz et al., 2010; Ohlendorf et al., 1986).

Aquatic consumers are primarily exposed to ash-derived Se through assimilation from diet (Franz et al., 2011; Luoma et al., 1992; Skorupa and Ohlendorf, 1991). Primary producers biotransform inorganic Se (usually selenite) into selenomethionine in their tissues (Alaimo et al., 1994), which is biologically available to primary consumers and readily transferred through food webs (Jarman et al., 1996; Unrine et al., 2007b). However, although Se is known to bioaccumulate through dietary exposure, its propensity to biomagnify (i.e., increase concentration via dietary uptake up three or more trophic levels; Dallinger et al., 1987) is less clear. The greatest enrichment of Se in aquatic food webs occurs during assimilation by primary producers (Presser and Luoma, 2010; Stewart et al., 2010). Enrichment of Se between subsequent trophic levels is dependent upon both its bioavailability in food and the assimilation efficiency of the consumer (Presser and Luoma, 2010). Thus, although Se concentrations are usually (but not always; Jardine and Kidd, 2011; Jarman et al., 1996; Unrine et al., 2007a) enriched between subsequent trophic levels (Ohlendorf et al., 1986; Presser and Luoma, 2010; Stewart et al., 2010), the magnitude of enrichment can differ among species within a given trophic level because of varying assimilation efficiency (Stewart et al., 2004). However, few studies of wild populations have traced among-species differences in Se bioaccumulation in higher consumers to among-species differences in primary consumers (Stewart et al., 2004).

Turtle species possess a suite of life history characteristics that make their assemblages useful for studying trophic influences on contaminant bioaccumulation (e.g., Bergeron et al., 2007; Congdon et al., 2008; W.A. Hopkins et al., 2013, Meyers-Schone and Walton, 1994). Turtles have small home ranges and long lifespans, and can be particularly susceptible to accumulating contaminants (Bergeron et al., 2007). Turtle species exhibit a diversity of dietary preferences (Ernst and Lovich, 2009), and are therefore likely to consume different prey types, with different body burdens of contaminants,

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within a contaminated area (Hopkins, 2000). As ectotherms, turtles can subsist on relatively small amounts of prey, and can reach much greater population sizes than endotherms occupying similar trophic levels (Iverson, 1982), which makes them relatively easy to sample.

In the current study, we examined the influence of feeding ecology on Se bioaccumulation in an assemblage of aquatic turtles inhabiting the Emory and Clinch River system in eastern Tennessee, USA. In December, 2008, 4.12 million cubic meters of coal fly-ash were accidentally discharged into the Emory–Clinch–Tennessee River system by the Tennessee Valley Authority's Kingston Fossil Plant (TVA, 2009). Subsequent remediation efforts removed the vast majority of ash prior to our study but ash-derived contaminants may still be entering local food webs. Following Bergeron et al. (2007) and W.A. Hopkins et al. (2013), we used stable isotopes (^{13}C and ^{15}N) from claws to test for among-species differences in relative carbon source and relative trophic positions. We then compared claw Se concentrations among species within the resulting trophic framework to determine whether relative carbon source and/or relative trophic position influenced bioaccumulation of ash-derived Se.

2. Methods

2.1. Sample collection

From April–July 2011, turtles were captured in the vicinity of the Kingston, TN Fossil Plant using hoop traps baited with sardines and/or chicken. All trapping occurred ~2.5 years after the spill event in December 2008 and ~1 year after the dredging efforts to remove ash from the river were completed in May 2010. Traps were set in shallow-water areas (<1 m deep) adjacent to microhabitats suitable for turtles. Trapping was concentrated in a contiguous 9.5 km length of river impacted by the fly-ash spill, including the Emory (river km 5.5–0.0) and Clinch Rivers (river km 7.0–3.0; Fig. 1). Traps were rebaited every 3 days, and were rotated among trapping locations depending upon trapping success. Captured turtles were removed from traps daily and transported to a field laboratory in Kingston, TN.

In the laboratory, turtle mass was measured with Pesola® scales, and carapace length, carapace width, and plastron length were measured using forestry calipers. The tips (top 2–3 mm) of all claws on the right rear foot (if present) were removed for analysis of Se, and the tips of all claws on the left rear foot (if present) were removed for stable isotope analysis. In several cases where turtles were missing all or a portion of a rear foot, the claw tips from the front feet were sampled instead. All turtles were released at the site of capture the day after processing. All claw samples were stored at −20 °C. We used claws because they can be sampled non-invasively, grow continuously, and exhibit very long tissue turnover rates (~12 mo.; Aresco, 2005). Therefore, claw stable isotope composition and Se concentration should represent a temporal integration of both diet and Se bioaccumulation over the previous year (Bearhop et al., 2003; W.A. Hopkins et al., 2013, 2007).

2.2. Turtle species

Claw stable isotope compositions and trace element concentrations were examined in seven species of turtles native to the Emory and Clinch Rivers near the site of the coal ash spill, including spiny softshell turtles (*Apalone spinifera*), snapping turtles (*Chelydra serpentina*), common map turtles (*Graptemys geographica*), Ouachita map turtles (*Graptemys ouachitensis*), common cooters (*Pseudemys concinna*), stinkpots (*Sternotherus odoratus*), and common sliders (*Trachemys scripta*). Although all of these freshwater turtles can be omnivorous to varying degrees, each differs in the relative proportions of prey types consumed (summarized from Ernst and Lovich, 2009). *Chelydra serpentina* can be highly piscivorous, and are likely to occupy the highest trophic position in most systems. *Apalone spinifera* are primarily carnivorous and are strongly sexually dimorphic in body size and diet; small males focus primarily on invertebrate prey, while large females are often more piscivorous. *Graptemys geographica* and *G. ouachitensis* are both medium-sized and feed primarily on mollusks. *Sternotherus odoratus* are small omnivores that focus on benthic invertebrates in soft-bottomed areas. Their benthic foraging strategy makes *S. odoratus* particularly susceptible to bioaccumulation of contaminants such as mercury (Bergeron et al., 2007). *Trachemys scripta* are a medium-sized dietary generalist that opportunistically feed on both plants and animals. *Pseudemys concinna* are primarily herbivorous, and likely occupy the lowest trophic position within this assemblage.

2.3. Stable isotope analysis

Stable isotope analysis followed the procedures established by Revesz and Qi (2006) and McCue and Pollock (2008). Claws sampled for stable isotope analysis were vortexed in millipore water to remove any external debris, and were dried to asymptotic mass at 50 °C. Dried claws were stored with Drierite® dessicant until isotopic analysis. At the University of Arkansas Stable Isotope Laboratory, 0.3–0.7 mg subsamples of claws were weighed on a Sartorius SC-2 nanobalance and wrapped in airtight 3 × 5 mm pressed tin capsules. If an entire set of claws weighed more than 0.7 mg, then claws were divided into subsamples which were individually analyzed and their values averaged together. Sealed sample-capsules were placed in a randomized order of analysis in 96-well microplates. All handling tools, surfaces, and weighing devices were cleaned with a methanol rinse and wiped with kimwipes after each sample. Standard reference materials (SRMs) were weighed and packaged in foil capsules in an identical manner, and were also added to the 96-well microplates. Six SRMs were added to the beginning of the microplate to precede all sample analyses. The first four SRMs were depleted in both ^{13}C and ^{15}N, while the latter two SRMs were enriched in both ^{13}C and ^{15}N. After the first 6 SRMs, claw samples were ordered in batches of nine, with every tenth sample being an additional SRM depleted in both ^{13}C and ^{15}N. An SRM depleted in both ^{13}C and ^{15}N was also added to the end of the microplate, and was the last sample analyzed on each microplate. A second microplate repeated the entire sequence. All microplates were sealed and stored in a dessicator until sample analysis.

Claw ^{15}N and ^{13}C contents were measured using a Finnigan Delta Plus continuous flow isotope ratio mass spectrometer (IR-MS) and elemental analyzer (EA). Samples sealed in tin cups were transferred from microplates and loaded in order into a microsampler on the EA. Under computer control, the autosampler dropped samples individually into a heated reaction tube in the EA, where they were combusted in a He atmosphere containing an excess of O_2 gas. Combustion converted total carbon and nitrogen from each sample into CO_2 and N_2 gas, respectively. From the EA, sample combustion gases were transported via He gas through a reaction furnace to remove excess O_2 gas and to convert any nitrous oxides into N_2, followed by a drying tube to remove water vapor. Carbon dioxide and N_2 gases were then separated by a gas chromatograph and introduced into the IR-MS through a Finnigan ConFlo II interface. The ConFlo II interface also introduces N_2 and CO_2 reference gases, and He gas for sample dilution. Mass and charge of the sample combustion gases measured by the IR-MS were uploaded to a PC and used to calculate $\delta^{13}C$ and $\delta^{15}N$ of each sample using Finnigan ISODAT 2.0 software. Isotopic determinations of $\delta^{13}C$ and $\delta^{15}N$ were normalized to a Vienna pee Dee belemnite standard and atmospheric N_2, respectively, using values provided by SRMs.

2.4. Selenium concentration analysis

Claw Se concentrations were quantified using Inductively Coupled Mass Spectrometry (ICP-MS) at the Trace Element Analysis Core at Dartmouth College. Claws were stored at 4° C prior to sample preparation and analysis. Claws were first washed to remove external contamination. Individual claws were transferred to a 7 ml polyethylene vial, 2 ml 1% solution of Triton X-100 was added and the vial was then placed in an ultrasonic bath for 20 min. The claw sample was washed 5 times with deionized water and then dried in the vial in a dry box. Each claw was then weighed into a pre-weighed VWR trace metal-clean polypropylene centrifuge tube and 0.5 ml of 9:1 HNO_3:HCl (Optima Grade, Fisher Scientific) was added. Individual claw weights were variable but were generally <0.025 g. Claws were prepared for acid digestion in batches of 100 samples along with five each of blank, certified reference material, and fortified blank quality control samples. The very small sample masses prevented digestion and analysis of matrix duplicates and matrix duplicate spikes. All tubes were lightly capped and placed into a CEM MARS Express (Mathews, NC) microwave digestion unit for an open vessel digestion. A fiber optic temperature probe was placed into one of the sample tubes to provide temperature feedback to the MARS unit and the samples were heated to 95 °C with a ramp to temperature of 15 min and held at temperature for 45 min. The samples were then allowed to cool and 0.1 ml of H_2O_2 (Optima Grade, Fisher Scientific) was added and the samples were taken through a further microwave heating program. The samples were then brought up to 10 ml with deionized water (Element QPod, Millipore, Billarica, MA). All measurements were recorded gravimetrically.

Digested samples were analyzed for Se by collision cell ICP-MS (7700×, Agilent, Santa Clara, CA). Selenium (78) was measured in hydrogen mode (2.8 ml min^{-1}) and Se (82) was measured in He mode (4.8 ml min^{-1}) along with other analytes. Analytical procedures followed the general protocols outlined in EPA 6020A; the instrument was calibrated with NIST-traceable standards and calibration was verified with a second source traceable standard. The reporting limits were checked after calibration before the analysis of each batch of samples. The instrument reporting limit was 0.08 μg/L and 0.3 μg/L for Se 78 and Se 82, respectively, corresponding to 0.095 mg/kg and 0.36 mg/kg dry mass average detection limits, respectively, for the claw samples. However, the detection limit for each claw was different and depended on the individual sample mass used in the digestion. Sample QC included continuing calibration verification and blanks every 10 samples, analytical duplicates and analytical spikes. Average recovery of certified reference material NIES Hair # 19, Se = 1.79 mg/kg was 104 ± 7% (n = 19), recovery of the fortified blank was 100 ± 11% (n = 19), average recovery of the analysis spiked samples was 105 ± 7% (n = 10) and relative percent difference of the sample analysis duplicates was 6 ± 6% (n = 10). The Se concentration of one *C. serpentina* sample was

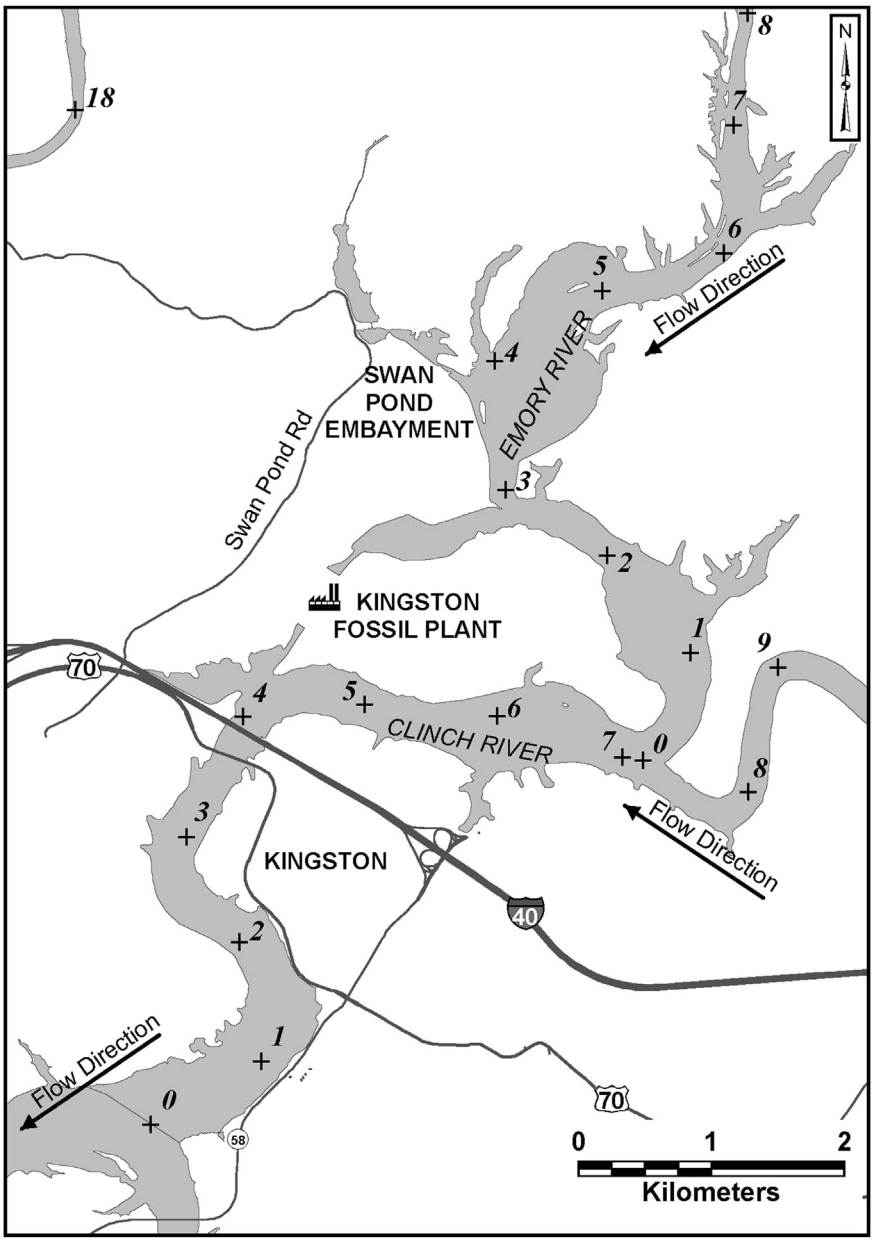

Fig. 1. Map of the turtle sampling area near Kingston, TN. Note that the Emory River is a tributary of the Clinch River, which is itself a tributary of the Tennessee River. Markers (+) and associated numbers indicate river kilometers, which are reported as distances from the downstream terminus of each river. The coal fly-ash spill of 22 December 2008 occurred at approximately Emory River kilometer 4, in and around the Swan Pond Embayment, which flows into the Emory River proper. All of the sampling reported in this study occurred from Emory River kilometers 5.5–0, and Clinch River kilometers 7–3 and represents a contiguous length of 9.5 river kilometers, approximately 8 of which are downstream of the spill site.

an order of magnitude greater than all other samples and was dropped from analysis because it was likely the result of a sampling error.

2.5. Statistical analysis

Turtle claw $\delta^{13}C$ and $\delta^{15}N$ values were compared among species using a multivariate analysis of covariance (MANCOVA; PROC GLM, SAS 9.2). Sex was included in the model as a main effect, while river was included as a random effect. Due to low sample sizes and similar feeding habits (Ernst and Lovich, 2009), the two congeneric map turtle species (*G. geographica* $N = 7$ and *G. ouachitensis* $N = 8$) were pooled together to improve statistical power. Turtle carapace length was included in the model as a covariate, because many turtles experience ontogenetic shifts in dietary preferences.

Among-species differences in claw Se concentration were examined using two approaches. First, ANCOVA was used to test for among-species differences in Se concentration, with carapace length, $\delta^{13}C$, and $\delta^{15}N$ as covariates. As in the isotope analysis, sex was included as a main effect, and river was included as a random effect. Both Se concentration and carapace length were log-transformed to improve normality. There were not enough degrees of freedom to test for significant differences in all 63 possible factors and interactions in a full factorial model, so we chose to examine the factorial interactions of all discrete factors (i.e., all interactive combinations of species, sex, and river), and all continuous factors (i.e., all interactive combinations of carapace length, $\delta^{13}C$, and $\delta^{15}N$). In addition, each possible bivariate interaction between discrete and continuous factors possible (e.g., species*carapace length, etc.) was examined to ensure that the assumption of slope homogeneity was met prior to ANCOVA. Second, ordinary least-squares regressions individually assessed the across-species relationships between $\delta^{13}C$ and Se concentration, and $\delta^{15}N$ and Se concentration. Selenium concentrations were log-transformed to improve normality in both regression models.

In all statistical tests, univariate normality and homoscedasticity of variance were assessed using normal probability plots and Shapiro–Wilk Tests. In all ANCOVAs, effects of the interactions between the covariate and main effects were examined before factor effects to ensure that the assumption of slope homogeneity

was met. Sample sizes were not large enough to test for violations of multivariate normality, so Pillai's Trace was used as a test statistic in all cases because it is the most robust to violations of multivariate normality (Scheiner, 2001). All statistical tests were judged at $\alpha = 0.05$, and all means are presented ±1 standard error (SE). All Se concentrations are reported on a dry mass.

3. Results

3.1. Turtles

During the 2011 trapping season, over 1000 turtles were captured, measured, and sampled. We compared claw Se concentrations, $\delta^{13}C$, and $\delta^{15}N$ from a subset comprised of 20 *A. spinifera*, 20 *C. serpentina*, 15 *Graptemys* sp. (7 *G. geographica* and 8 *G. ouachitensis*), 15 *P. concinna*, 19 *S. odoratus*, and 20 *T. scripta*.

3.2. Stable isotopes

Turtle claw isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) varied significantly with carapace length and with the interaction of river, sex, and species in the overall multivariate model (Table 1). To understand the causes of this complex interaction, the effects of each factor on $\delta^{13}C$ and $\delta^{15}N$ were examined in post-hoc univariate analyses. Mean $\delta^{13}C$ varied significantly with the interaction among river, sex, and species (Table 2) and was likely responsible for the significant interaction in the multivariate model. This significant interaction was driven primarily by male *P. concinna* from the Emory River (−19.07 ± 1.22‰), which were significantly more enriched in $\delta^{13}C$ relative to all other river–sex–species combinations (Tukey Test $p < 0.001$). The only other within-species differences observed were in *A. spinifera*, where mean $\delta^{13}C$ was significantly more enriched in males from the Clinch River (−27.44 ± 0.99‰) than in males from the Emory River (−30.10 ± 0.71‰; Tukey Test $p = 0.031$), and in *T. scripta*, where mean $\delta^{13}C$ was significantly more enriched in females from the Emory River (−24.35 ± 0.55‰) than in either males (−30.12 ± 1.74‰) or females (−27.73 ± 0.77‰) from the Clinch River (Tukey Test $p \leq 0.002$ in both cases). Overall, mean $\delta^{13}C$ varied significantly among species (Table 2; Fig. 2), and in the following fashion: *P. concinna* > *T. scripta* = *S. odoratus* = *C. serpentina* ≥ *A. spinifera* > *Graptemys*.

Mean $\delta^{15}N$ increased with carapace length in all species (Tables 1 and 2). Mean $\delta^{15}N$ also varied significantly among species (Table 2; Fig. 2), in the following fashion: *C. serpentina* = *Graptemys* sp. > *A. spinifera* = *T. scripta* = *S. odoratus* ≥ *P. concinna*. In addition to species differences, mean $\delta^{15}N$ was significantly higher in turtles from the Clinch River than in turtles from the Emory River (Table 2). However, mean $\delta^{15}N$ did not differ between males and females, and no interactions among factor effects were significant (Table 2).

Table 1
Results of MANCOVA analysis of mean claw $\delta^{13}C$ and $\delta^{15}N$ sampled from turtles from the vicinity of the coal fly-ash spill in the Emory and Clinch Rivers, TN. The interaction between carapace length, river, sex, and species tests the assumption of homogeneity of slope necessary to ANCOVA, and no other interactions with carapace length were examined. Error degrees of freedom was 114 in all cases, and asterisks indicate factors significant at $\alpha = 0.05$.

Source	Pillai's Trace	*F*	*df*	*p*
Carapace length*	0.161	7.49	2	0.001
River*	0.193	9.32	2	<0.001
Sex	0.037	1.52	2	0.226
Species*	0.738	9.23	10	<0.001
River × sex	0.042	1.71	2	0.187
River × species*	0.347	3.31	10	<0.001
Sex × species	0.175	1.52	10	0.137
River × sex × species*	0.233	2.08	10	0.029
Carapace length × river × sex × species	0.077	0.57	8	0.800

Table 2
Results of post-MANCOVA univariate analyses of claw $\delta^{13}C$ and $\delta^{15}N$ sampled from turtles from the vicinity of the coal fly-ash spill in the Emory and Clinch Rivers, TN. Error degrees of freedom was 103 in all cases, and asterisks indicate factors significant at $\alpha = 0.05$.

Source	*F*	*df*	*p*
$\delta^{13}C$			
Carapace length	1.83	1	0.180
River*	8.17	1	0.005
Sex	0.88	1	0.351
Species*	19.39	5	<0.001
River × sex	2.94	1	0.090
River × species*	5.31	5	<0.001
Sex × species*	2.66	5	0.028
River × sex × species*	3.85	5	0.004
$\delta^{15}N$			
Carapace length*	12.14	1	<0.001
River*	12.70	1	<0.001
Sex	2.47	1	0.120
Species*	7.62	5	<0.001
River × sex	0.83	1	0.366
River × species	2.07	5	0.078
Sex × species	0.42	5	0.835
River × sex × species	1.29	5	0.759

3.3. Selenium concentration

Turtle claw Se concentrations did not vary with any of the interactions examined (all two and three way interactions from ANCOVA: $F \leq 3.72$ $p \geq 0.059$). Claw Se concentration varied significantly among species ($F_{5, 107} = 15.28$, $p < 0.001$), but did not vary with carapace length ($F_{1, 107} = 0.18$, $p = 0.669$), with $\delta^{13}C$ ($F_{1, 107} = 0.18$, $p = 0.676$), with $\delta^{15}N$ ($F_{1, 107} = 0.19$, $p = 0.667$), between rivers ($F_{1, 107} = 0.45$, $p = 0.503$), or between sexes ($F_{1, 107} = 1.49$, $p = 0.232$). Across species, mean claw Se concentrations were significantly higher in *A. spinifera* than in all other species (Fig. 3; Tukey Test $p < 0.001$), but did not differ among any other species examined (Fig. 3; Tukey Test $p \geq 0.444$). While ANCOVA did not reveal any significant relationships between claw Se concentration and claw $\delta^{13}C$ or $\delta^{15}N$ within species, ordinary least squares regression did reveal that Se concentrations significantly decreased with increasing $\delta^{13}C$ across species (Fig. 4A; $F_{1, 107} = 31.14$, $p < 0.001$). However, there was no significant relationship between $\delta^{15}N$ and claw Se concentration (Fig. 4B; $F_{1, 107} = 0.70$, $p = 0.406$).

4. Discussion

Claw Se concentrations significantly differed among species within our turtle assemblage. Because Se is assimilated primarily from diet (Luoma et al., 1992), Se concentration differences likely reflect differences in dietary preference. Our stable isotope results suggest that the species we studied significantly differ in their dietary preferences. However, Se concentrations differed only with relative carbon source, and not with relative trophic position. Here, we describe the trophic differences observed in our turtle assemblage, and then use the resulting trophic framework to interpret among-species differences in Se concentration.

4.1. Stable isotopes

Stable isotope analyses generally supported the known dietary preferences of turtle species in our study. The ranges in individual $\delta^{15}N$ values (6.2–16.8‰) and mean species $\delta^{15}N$ values (8.3–13.2‰) suggested that turtles were feeding at more than one trophic level, assuming a 2–5‰ increment between successive trophic levels (Peterson and Fry, 1987; Post, 2002; Vander Zanden and Rasmussen, 2001). *Chelydra serpentina* and *Graptemys* sp.

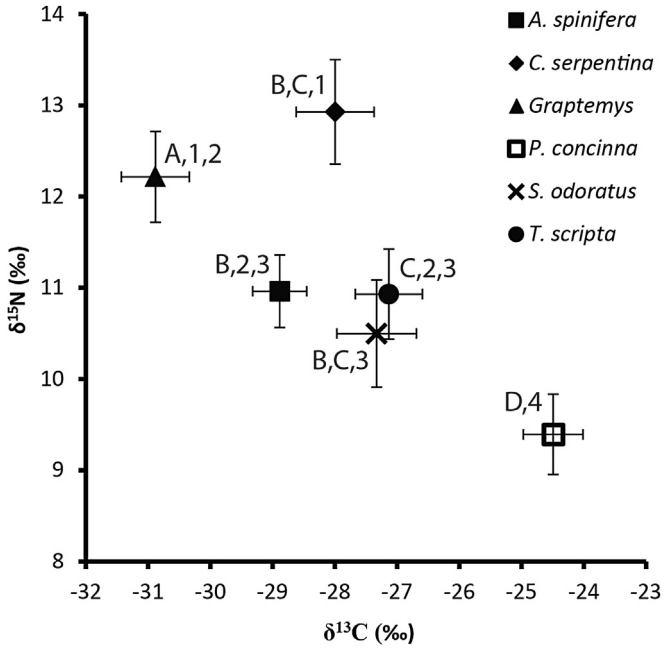

Fig. 2. Isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of turtle claws sampled from turtle species inhabiting Emory and Clinch Rivers near the site of the Kingston coal fly-ash spill. Both $\delta^{13}C$ and $\delta^{15}N$ varied significantly among species. Symbols represent least-squares means for each species corrected for carapace length. Significant differences among rivers and/or sexes are not shown for simplicity. Letters indicate significant differences among species in $\delta^{13}C$ (Tukey Test $p \leq 0.015$ for significant differences, $p \geq 0.074$ for non-significant differences), while numbers indicate among-species significant differences in $\delta^{15}N$ (Tukey Test $p \leq 0.023$ for significant differences, $p \geq 0.063$ for non-significant differences). Error bars represent ±1 SE.

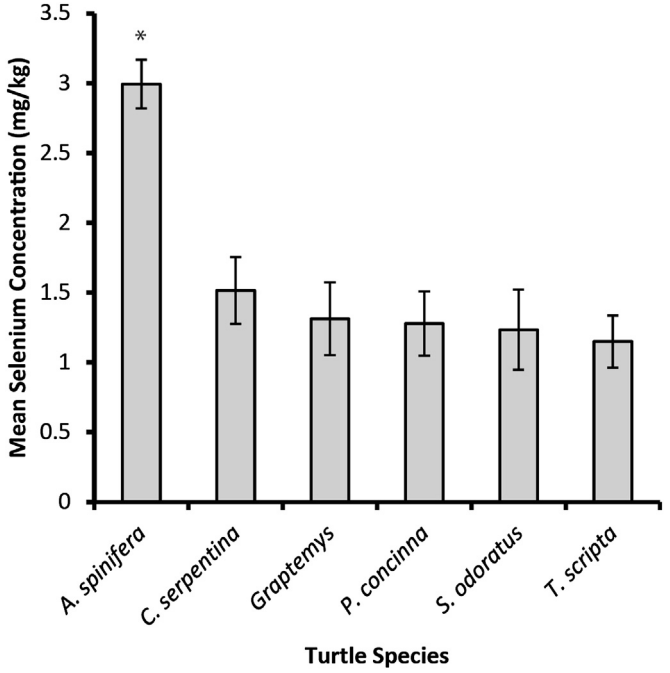

Fig. 3. Claw Se concentrations (mg/kg dry mass) sampled from turtle species inhabiting Emory and Clinch Rivers near the site of the Kingston coal fly-ash spill. Mean Se concentrations of turtle claws were significantly higher in *Apalone spinifera* than in all other species. Error bars represent ±1 SE and the asterisk represents a significant difference.

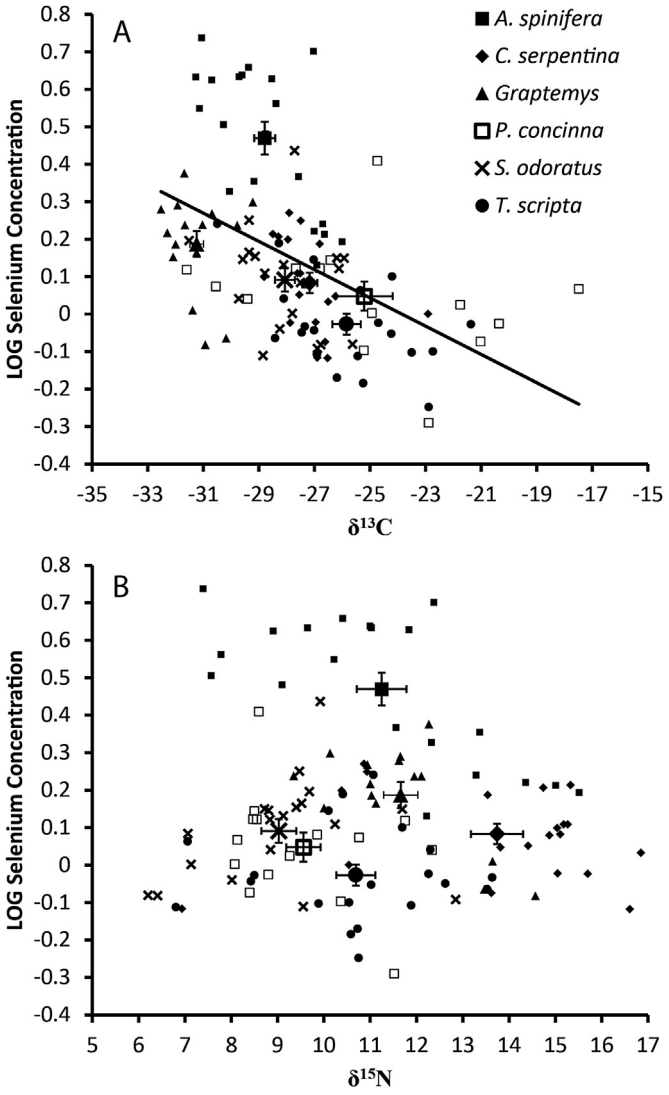

Fig. 4. Log-transformed Se concentrations (mg/kg dry mass) are shown regressed against $\delta^{13}C$ (A) and $\delta^{15}N$ (B). Small symbols represent values for individual turtles, while larger symbols with error bars represent species means ±1 SE. The relationship between log-transformed Se concentration and $\delta^{13}C$ was significant, and is best explained by the equation: $LOG(Se) = -0.038(\delta^{13}C) - 0.906$ ($F_{1,\ 107} = 31.14$ $p < 0.001$; $r^2 = 0.21$). There was no significant relationship between log-transformed Se concentration and $\delta^{15}N$ ($F_{1,\ 107} = 0.23$ $p = 0.602$).

exhibited the most enriched $\delta^{15}N$, suggesting that they were feeding on the highest trophic level. *Chelydra serpentina* are primarily carnivorous and large individuals often eat fish, which can occupy relatively high trophic levels themselves (Ernst and Lovich, 2009), so their placement at the highest trophic level is not surprising. *Graptemys* are highly carnivorous as well but typically feed on bivalves (Ernst and Lovich, 2009). *Pseudemys concinna* exhibited the most depleted $\delta^{15}N$ values (9.4‰), which suggests that they were feeding on the lowest relative trophic position. *Pseudemys concinna* is primarily herbivorous (Ernst and Lovich, 2009), so its placement at the lowest trophic level is consistent with its known feeding ecology.

Relative to the extremes mentioned above, *A. spinifera*, *S. odoratus*, and *T. scripta* exhibited intermediate and overlapping $\delta^{15}N$ values. *Apalone spinifera* and *S. odoratus* feed primarily on invertebrates (Ernst and Lovich, 2009), and would be expected to overlap with each other. However, it is surprising that their mean $\delta^{15}N$ values (10.9‰ and 10.6‰, respectively) were significantly

depleted relative to those of *Graptemys*, which also primarily feed on invertebrates. The discrepancy could reflect differences in dietary specialization; *Graptemys* often specialize on bivalves, while *A. spinifera* and *S. odoratus* often feed more broadly on a diversity of invertebrates (Ernst and Lovich, 2009). *Trachemys scripta* is likely the most generalist species in our study, and its intermediate overall mean $\delta^{15}N$ (10.9‰) could be due to its propensity to feed opportunistically on both plant and animal matter.

In addition to the observed species differences, $\delta^{15}N$ was significantly higher in all turtles from Clinch River than from the Emory River, regardless of species. This result is somewhat surprising because our study site comprises a fairly small area (Fig. 1), and preliminary mark-recapture data suggest that individuals of some species are capable of moving between rivers (Steen et al., unpublished data). Given the long time required for claw tissue turnover in turtles ($\sim$12 mo.; Aresco, 2005), our data suggest that even if individual turtles travel between the Emory and Clinch Rivers, the majority of their feeding may be restricted to only one river. Ultimately, the between-river difference in $\delta^{15}N$ suggests either that turtles feed at slightly different trophic positions in the Emory and Clinch Rivers, or that relative baseline nitrogen isotopic enrichment differs between the two rivers. The latter seems more likely because the Clinch River drains a much larger watershed than the Emory River (including the Emory itself) and has a greater potential for anthropogenic disturbance (Atchley et al., 2000; Burr et al., 2000), both of which may increase $\delta^{15}N$ in aquatic primary producers (Cabana and Rasmussen, 1996; March and Pringle, 2003).

As with $\delta^{15}N$, we also found differences in $\delta^{13}C$, both among species and between rivers. ^{13}Carbon does not fractionate with successive trophic levels, but differs with producer photosynthetic pathway (C_3, C_4), and has been used to differentiate the ultimate sources of carbon among species (Peterson and Fry, 1987; Post, 2002). Individual $\delta^{13}C$ values ranged from -32.5 to -17.5‰, and species means ranged from -30.7 to -23.5‰. Overall, our results suggested that *P. concinna* were feeding on more enriched carbon sources than all other species, while *Graptemys* were feeding on more depleted carbon sources, and *A. spinifera*, *C. serpentina*, *S. odoratus*, and *T. scripta* were feeding on carbon sources that were intermediate in carbon enrichment. These differences likely reflect different rates of consumption of C_3 and C_4 producers at the bases of food chains utilized by different species (Peterson and Fry, 1987; Post, 2002).

4.2. Selenium

Although our $\delta^{15}N$ results suggest that turtles in the Emory–Clinch River system are feeding at different trophic levels, we observed no relationship between relative trophic position and Se concentration. Selenium concentrations did not change consistently with relative trophic position; therefore, we found no evidence of biomagnification of Se in this turtle assemblage. Indeed, the species at the highest trophic levels, *C. serpentina* and *Graptemys*, exhibited Se concentrations similar to the species at the lowest trophic level, *P. concinna. Apalone spinifera*, which fed at an intermediate trophic level, exhibited significantly higher Se concentrations than all other species. Furthermore, *S. odoratus* and *T. scripta* fed at intermediate trophic levels similar to that of *A. spinifera*, but did not exhibit elevated Se concentrations. Thus, our findings suggest that *Apalone spinifera* consume different food items at that trophic level, which are relatively enriched in Se. Similarly, Orr et al. (2006) found that invertebrate species feeding at identical trophic positions exhibited highly variable Se concentrations. Notably, there are diverse invertebrate and vertebrate primary and secondary consumers present in the Tennessee River system, including bivalves, gastropods, insects, fish, and larval amphibians. Among-taxa differences in Se enrichment within this assemblage could explain our observation. Alternatively, if Se concentrations are not different among turtle prey at a given trophic level, our results might suggest that Se assimilation efficiencies differ among turtle species, as has been observed in some invertebrates (Stewart et al., 2004). Another possibility is that turtles exhibiting enriched $\delta^{13}C$ may be relying more heavily on terrestrial carbon sources (Willson et al., 2010), which may be low in Se concentrations if the primary producers are above the water line and thus outside of areas contaminated by the fly-ash spill. Regardless of the underlying explanation, our work corroborates prior studies on other taxonomic groups that reported a lack of evidence for Se biomagnification among consumers at different relative trophic levels (Jardine and Kidd, 2011; Jarman et al., 1996; Ohlendorf et al., 1986; Orr et al., 2012; Unrine et al., 2007a).

Selenium concentrations decreased with increasing $\delta^{13}C$ across all turtle species, suggesting that Se bioaccumulation differed with relative carbon source. Importantly, the relationship remained significant even if *A. spinifera* were removed from the analysis ($P < 0.001$). Jardine and Kidd (2011) suggested that Se concentrations might change with $\delta^{13}C$ if $\delta^{13}C$ were considered a proxy for distance from a Se contamination site. In our system, $\delta^{13}C$ differed between rivers only within *A. spinifera*, *S. odoratus*, and *T. scripta*, but we found no evidence for within-species relationships between $\delta^{13}C$ and Se concentration. Therefore, it seems unlikely that the Se differences we observed were due to site differences in Se contamination in any species in our study. Alternatively, turtle Se concentrations might be influenced by variable Se assimilation or retention rates in different primary producers. All aquatic producers are thought to assimilate Se with relative ease (Ornes et al., 1991; Stewart et al., 2010), but the differences in turtle Se concentrations and relative carbon source could indicate that aquatic producers that are depleted in ^{13}C might concentrate Se at a greater rate than do producers that are enriched in ^{13}C. Littoral periphyton and aquatic plants are typically enriched in ^{13}C relative to benthic algae and phytoplankton (France, 1995a, b). Therefore those turtles exhibiting high Se concentrations and depleted ^{13}C may be feeding predominately from food webs originating from benthic algae and phytoplankton, while those exhibiting low Se concentrations and enriched ^{13}C may be feeding predominately from food webs originating from periphyton and aquatic plants. Variable Se trophic transfer rates between different species of primary producers and primary consumers likely also play a role in the observed pattern (Stewart et al., 2010). While Se concentration data are unavailable for algae, Se concentrations in periphyton are 1–2 orders of magnitude higher than those of aquatic and emergent plants at the Kingston spill site (Arcadis, 2012). Furthermore, Se concentrations were higher in mayfly nymphs than in aquatic snails (Smith, 2012), both of which are likely prey species for turtles at the site. These data support our hypothesis that differences in Se bioaccumulation among primary producers that differ in ^{13}C enrichment, as well as subsequent differences among primary consumers, could affect Se bioaccumulation in higher consumers like turtles.

Although mean Se concentrations in *A. spinifera* were higher than those of other species, the relationship between claw Se concentration and health effects have not been determined in any vertebrate. Thus, the toxicological significance of the Se concentrations reported here is unknown. Preliminary analyses suggest that claw Se concentrations are correlated with the Se concentrations of blood and muscle (Van Dyke et al., unpublished data), as has been described for other contaminants in turtles (B.C. Hopkins et al., 2013; W.A. Hopkins et al., 2013). Forthcoming examinations of turtle reproductive success will determine whether the turtles in this system face any adverse consequences of Se bioaccumulation. In addition, forthcoming examinations of temporal effects on Se

concentrations will determine whether remediation efforts successfully averted further Se bioaccumulation in turtles.

5. Conclusions

Our results demonstrated that relative trophic position does not predict Se bioaccumulation in an assemblage of closely-related vertebrates exposed to a recently remediated coal fly-ash spill. Our results contribute to a growing body of evidence that Se does not consistently biomagnify in higher-order consumers (Jardine and Kidd, 2011; Jarman et al., 1996; Ohlendorf et al., 1986; Orr et al., 2012; Unrine et al., 2007a). However, Se concentrations did decrease with increasing $\delta^{13}C$ across all species. Delta-^{13}C is determined by ^{13}C fractionation rates during photosynthesis in primary producers at the base of the food web (Peterson and Fry, 1987; Post, 2002). Therefore, our results suggest that Se may be more bioavailable to turtles feeding on food webs in which aquatic producers are depleted in $\delta^{13}C$ (i.e., benthic algae and phytoplankton) than to those feeding on food webs in which producers are enriched in $\delta^{13}C$ (i.e., littoral periphyton and aquatic plants). Although Se bioaccumulation rate is known to vary among primary producers (Stewart et al., 2010), prior studies of trophic enrichment have focused on food webs based on a single primary producer species (Besser et al., 1993; Conley et al., 2009; Orr et al., 2012; Stewart et al., 2004), or have not been able to differentiate species differences from abiotic effects associated with differences in preferred habitat (Orr et al., 2006). Thus, the possibility that variability in Se bioaccumulation among syntopic producer species might contribute to differences in Se exposure at higher trophic levels has been relatively unexplored. Elucidating these relationships is critical to advancing our understanding of Se trophic dynamics (B.C. Hopkins et al., 2013).

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