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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