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ELEMENT LEVELS IN SNAKES IN SOUTH CAROLINA: DIFFERENCES BETWEEN A CONTROL SITE AND EXPOSED SITE ON THE SAVANNAH RIVER SITE

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Abstract. Levels of 18 elements, including lead, mercury, selenium, and uranium, were examined in three species of snakes from an exposed and reference site on the Department of Energy's Savannah River Site in South Carolina. We tested the hypotheses that there were no differences as a function of species, and there were no difference between the exposed and control site for blood and muscle (tail) samples for banded water snake (*Nerodia fasciata*), brown water snake (*N. taxispilota*) and cottonmouth (*Akistrodon piscivorous*). The banded water snakes collected were significantly smaller than the other two species. For blood, there were significant species differences only for barium, copper, selenium, uranium and zinc, while for muscle tissue there were significant interspecific differences in aluminum, arsenic, barium, cobalt, cesium, copper, iron, lead, mercury, manganese, strontium, vanadium and zinc, suggesting that muscle tissue in the tail is a better indicator of potential interspecific differences. It is also easier logistically to collect tail tissue than blood. Where one species had significantly higher levels than the other species in muscle tissue levels, cottonmouth had higherlevels of five elements (aluminum, cobalt, lead, mercury, vanadium), brown water snake had two (lead, strontium), and banded water snake had only barium. There were few significant differences between the control and reference site for levels of blood, but several for muscle tissue. All three species had significantly higher levels of arsenic and manganese at Tim's Branch than the reference site, and nickel and uranium were significantly higher for banded watersnake and cottonmouth, the larger species. Individuals with high exposure of one element were exposed to high levels of other elements.

Keywords: bioindicators, cottonmouth, elements, heavy metals, water snakes

1. Introduction

Elements enter the food chain through industrial sources, natural erosion, and biogeochemical cycles. Elemental loads are a result of uptake minus elimination, bearing in mind that some trace elements are internally regulated (e.g. cobalt, chromium, copper, iron, manganese). Different animals bioconcentrate elements to different degrees (Barron, 1995; Burger *et al.*, 2001a, 2002). While global transport and deposition is an important source of elemental exposure for organisms (Fitzgerald, 1989), anthropogenic contaminants from urban, industrial and agricultural runoff are major sources as well (Mailman, 1980). This is particularly true with sites contaminated in the past where many environmental protection laws were not yet in effect, or industries were not held accountable. Finding suitable bioindicators of exposure in such systems is particularly critical to decisions about cleanup and future land use (Linthurst *et al.*, 1995; Burger and Gochfeld, 2001).

While considerable attention has been devoted to examining the patterns of heavy metal and other element accumulation in closely related groups of fish and of birds in aquatic and terrestrial systems (Burger, 2002; Burger *et al.*, 2002), little attention has been devoted to closely related reptiles within the same ecosystem (Andreadis, 1998; Campbell and Campbell, 2001). Yet snakes are often top-level predators that might shed insight into both bioaccumulation, species differences, and bioindicator selection (Bauerle, 1975; Ernst and Barbour, 1989; Hopkins *et al.*, 2001; Campbell and Campbell, 2001; Burger *et al.*, in press).

In this paper we examine the levels of 18 elements (including some heavy metals and radionuclides) in two tissues (blood, tail tissue) of three species of snakes from a control and reference site on the Department of Energy's Savannah River Site. All three species of snakes are largely aquatic, and included banded water snake (*Nerodia fasciata*), brown water snake (*N. taxispilota*) and cottonmouth (*Akistrodon piscivorous*). We test the hypotheses that: (1) there are no interspecific differences in element levels, (2) there are no locational differences in element levels between the exposed (Tim's Branch) and reference site, and (3) there are no consistent relationships among elements. We were particularly interested in whether one of the three species consistently had the highest levels, suggesting that they may be the best bioindicator of environmental contamination, and whether blood of tail tissue would show the greatest differences. We expected there to be differences between the exposed and reference sites (after Punshon *et al.*, 2003; Murray, 2003).

There are few reports of element levels in snakes, although there is more information from water snakes than for other species groups (Campbell and Campbell, 2001). Levels of organochlorine pesticides, polychlorinated biphenyls and some metals (copper, mercury, selenium, zinc) were examined in blood of diamondback water snakes (*Nerodia rhombifer)* and blotched water snakes (*Nerodia erythrogaster*) in Texas (Clark *et al.*, 2000), pesticides in water snakes and cottonmouths from Mississippi (Ford and Hill, 1991), metal concentrations in banded water snakes (*Nerodia fasciata)* from the SRS (Hopkins *et al.*, 2001), PCBs and organochlorine pesticides in northern water snakes and Lake Erie water snakes (*Nerodia sipedon insularum*) from the Great Lakes Basin (Bishop and Rouse, 2000), and heavy metals in northern water snakes (*Nerodia sipedon*) from Tennessee (Burger *et al.*, in press). None of these studies examined three species of aquatic snakes in contaminated and reference sites in both blood and tail snips, although Clark *et al.* (2000) also examined levels in cottonmouths. Blood and tail clips are those being considered for non-destructive bioindicators (see Hopkins *et al.*, 2001; Burger *et al.,* in press).

2. Study Sites and Methods

2.1. Study sites

The Department of Energy's Savannah River Site (SRS) is a 780 km^2 former nuclear production and current research facility located adjacent to the Savannah River in South Carolina. Georgia is across the river from the site (Figure 1). In the early 1950s SRS was established as a nuclear weapons production facility, and several contaminants have been released on site (Kvartek *et al.*, 1994; Sugg *et al.*, 1995).

Tim's Branch, part of the Upper Three Runs watershed that drains into the Savannah River swamp system, was contaminated by chemical and radiation releases in the period from the 1950s to the 1980s, and residual contamination remains today. The Steed's Pond/Tim's Branch depositional system, on the northwestern corner of SRS, was impacted by discharges from the 1950s to the 1980s by discharges of depleted and natural uranium, aluminum, nickel, copper, chromium, zinc, lead, and other associated metals (Bertsch *et al.*, 2000). Approximately 44,000 kg of depleted uranium was released into Steed's Pond (Pickett, 1990).

2.2. Methods

We collected three species of aquatic snakes from the Steed's Pond/Tim's Branch depositional system and from two reference sites (Boggy Gut and Upper Three Runs), located upstream from the contaminated sites within the same watershed from April to late October 2001, and from March through July 2002. These snakes hibernate from November through February and are inaccessible then.

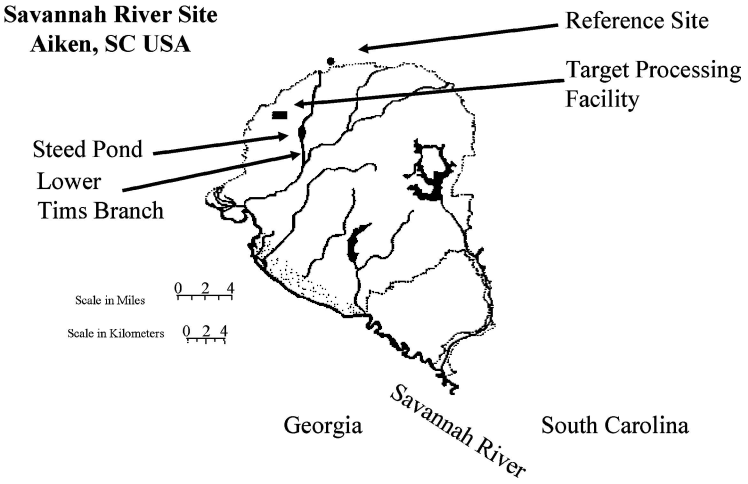

Figure 1. Map of the Department of Energy's Savannah River Site showing all study sites.

Minnow traps baited with small fish were used to trap the snakes, and were checked daily. Snakes were weighed, measured (snout-vent length), sexed by probing techniques, and were taken to the on-site Savannah River Ecology Laboratory where blood and muscle samples were taken. We drew 1–3 cc of blood from the caudal vein below the vent, using a 28 gauge needle, and clipped 8–10 mm of the tail tip representative of whole body tissue. There are fewer blood samples because it was impossible to obtain enough blood from some of the snakes.

After collection, blood and muscle tissue samples were freeze-dried and digested with 5 ml 5 M HNO_3 (trace metal grade) in pure Teflon PFA vessels using microwave digestion (MDS-2000, CEM Corporation, Mathews, NC). A HNO_3 blank and a NIST standard reference material (TORT) were included with each carousel, after which samples were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS).

Ni concentrations in prey items were determined in the same way as the blood and tail snips. Animals were freeze-dried, digested using the whole body to reflect what the snake ingests, and analyzed using ICP-MS. Digested tissue samples followed a modified methodology outlined in EPA method 3052 and quality control procedures based on EPA 6020. The ICP-MS was calibrated using Custom Grade standards at the beginning of each batch, and after every fifteenth sample. All concentrations are expressed in parts per million (ug/g) on a wet weight basis. Detection limits in ug/g were Al = 185, As = 36, Ba = 103, Co = 111, Cu = 129, Cr = .183, Cs = 109, Pb = 102, Mn = 114, Hg = 46, Ni = 183, Rb = 114, Se = 11, St^{88} = 97, U = 126, and Zn = 123. The contaminants of particular concern (COPC) from Tim's Branch are U, Ni, Cu, Zn, although other metals have also been found to correlate with these contaminants of concern (Punshon *et al.*, 2003).

An EPA standard was run at the beginning of each batch for initial calibration verification. All specimens were run in batches that included blanks, a standard calibration curve and spiked specimens. The accepted recoveries for spikes ranged from 80 to 120%; no batches were outside of these limits. The coefficient of variation (C.V.) on replicate samples ranged from 2 to 8%.

We used Kruskal–Wallis one way analysis of variance to examine for differences among locations (SAS, 1995). The level for significance was designated as <0.05, but values between this level and 0.1 are presented to allow the reader to evaluate whether increased sample sizes would have resulted in significance.

3. Results

3.1. INTERSPECIFIC COMPARISONS

Banded water snakes were shorter and lighter than the other snakes collected (Table I). Although the interspecific differences in snout-vent length were small, cottonmouths were twice as heavy as banded water snakes. We were not able to collect

TABLE I
Overall means and standard error of metals in banded, cottonmouth, and brown water snakes collected from the Department of Energy site in South Carolina. Below are geometric means with duncan values

	Banded water snake	Brown water snakes	Cotton mouth	$X^2(p)$
Sample size	47	10	13	
Snout-vent length (cm)	51 ± 2	59 ± 3	57 ± 4	6 (0.05)
	49 (A)	57 (A)	56 (A)	
Mass (g)	130 ± 16	170 ± 33	280 ± 35	13 (0.002)
Muscle tissue	100	140	240	
Aluminum	93 ± 20	169 ± 34	431 ± 89	24 (0.0001)
	47 (B)	124 (B)	323 (A)	
Arsenic	0.40 ± 0.04	0.7 ± 0.09	7.00 ± 0.09	16 (0.0003)
	0.3 (B)	0.6 (A)	0.6 (A)	
Barium	64 ± 4	28 ± 3	43 ± 4	32 (0.0001)
	50 (A)	24 (C)	41 (B)	
Cobalt	0.24 ± 0.02	0.2 ± 0.02	0.31 ± 0.03	6 (0.05)
	0.2 (A,B)	0.2 (B)	0.3 (A)	
Chromium	0.9 ± 0.2	1 ± 0.1	1 ± 0.3	5 (0.08)
	0.6 (A)	0.4 (A)	0.7 (A)	
Cesium	0.3 ± 0.02	0.2 ± 0.02	0.3 ± 0.03	6 (0.04)
	0.2 (A)	0.14 (A)	0.2 (A)	
Copper	2 ± 0.2	3 ± 0.4	3 ± 0.3	17 (0.0002)
	1 (B)	2 (A, B)	3 (A)	
Iron	663 ± 72	1779 ± 361	1527 ± 274	22 (0.0001)
	499 (B)	1210 (A)	1257 (A)	
Lead	2 ± 0.2	32 ± 0.5	3 ± 1	10 (0.006)
	1 (B)	3 (A)	2 (A, B)	
Mercury	0.6 ± 0.05	0.7 ± 0.1	0.9 ± 0.1	6 (0.06)
	0.5 (B)	0.5 (B)	0.8 (A)	
Manganese	66 ± 6	88 ± 10	19 ± 5	20 (0.0001)
	41 (A)	74 (A)	12 (B)	
Nickel	5 ± 1	5 ± 1	5 ± 1	NS
	4 (A)	3 (A)	4 (A)	
Selenium	1 ± 0.09	2 ± 0.4	1 ± 0.2	NS
	1 (A)	1 (A)	1 (A)	
Rubidium	26 ± 1	21 ± 2	21 ± 2	NS
	21 (A)	18 (A)	25 (A)	

(*Continued on next page*)

TABLE I
(*Continued*)

	Banded water snake	Brown water snakes	Cotton mouth	$X^2(p)$
Strontium	94 ± 4	156 ± 13	152 ± 14	39 (0.0001)
	70 (B)	137 (A)	48 (C)	
Uranium	1 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	NS
	0.3 (A)	0.4 (A)	0.3 (A)	
Vanadium	0.6 ± 0.1	0.9 ± 0.1	2 ± 1	13 (0.001)
	0.4 (B)	0.7 (B)	1 (A)	
Zinc	150 ± 9	151 ± 12	141 ± 11	12 (0.002)
	123(A)	128 (A)	90 (B)	
Blood	$n = 34$	$n = 9$	$n = 5$	
sv	52 ± 2	65 ± 3	60 ± 5	9 (0.01)
	51 (A)	64 (A)	60 (A)	
Mass	140 ± 19	230 ± 48	270 ± 48	11 (0.003)
	108 (B)	200 (A,B)	250 (A)	
Aluminum	2 ± 0.3	2 ± 0.5	3 ± 2	NS
	1 (A)	1 (A)	1 (A)	
Arsenic	0.3 ± 0.03	0.4 ± 0.08	0.3 ± 0.10	NS
	0.1 (A)	0.2 (A)	0.2 (A)	
Barium	0.4 ± 0.02	0.3 ± 0.03	0.6 ± 0.2	10 (0.006)
	0.4 (B)	0.3 (B)	0.5 (A)	
Cobalt	0.1 ± 0	0.08 ± 0.03	0.1 ± 0.01	NS
	0.06 (A)	54 (A)	0.06 (A)	
Chromium	0.6 ± 0.04	0.1 ± 0.02	0.1 ± 0.04	NS
	0.1 (A)	0.08 (A)	0.07 (A)	
Cesium	0.03 ± 0.005	0.04 ± 0.01	0.05 ± 0.02	NS
	0.02 (A)	0.03 (A)	0.03 (A)	
Copper	0.6 ± 0.04	0.3 ± 0.04	0.6 ± 0.1	11 (0.003)
	0.5 (A)	0.3 (B)	0.6 (A)	
Iron	236 ± 12	220 ± 28	190 ± 39	NS
	222 (A)	199 (A)	168 (A)	
Lead	0.1 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	NS
	0.03 (A)	0.03 (A)	0.03 (A)	
Mercury	0.4 ± 0.05	0.7 ± 0.15	0.1 ± 0.04	8 (0.02)
	0.3 (A,B)	0.5 (A)	0.1 (B)	
Manganese	0.17 ± 0.02	0.2 ± 0.03	0.12 ± 0.06	NS
	.15 (A)	0.2 (A)	0.07 (A)	
Nickel	0.2 ± 0.03	0.1 ± 0.02	0.2 ± 0.1	NS
	0.1 (A)	0.1 (A)	0.1 (A)	

(*Continued on next page*)

TABLE I
(*Continued*)

	Banded water snake	Brown water snakes	Cotton mouth	$X^2(p)$
Selenium	0.4 ± 0.03	1 ± 0.09	0.6 ± 0.2	15 (0.0007)
	0.4 (B)	0.8 (A)	0.5 (A,B)	
Rubidium	5 ± 0.3	4 ± 1	3 ± 0.2	NS
	4 (A)	4 (A)	3 (A)	
Strontium	0.14 ± 0.01	0.2 ± 0.03	0.2 ± 0.03	NS
	0.13 (A)	0.2 (A)	0.2 (A)	
Uranium	0.04 ± 0.01	0.07 ± 0.02	0.06 ± 0.03	5 (0.07)
	0.02 (A)	0.04 (A)	0.03 (A)	
Vanadium	0.04 ± 0.004	0.04 ± 0.01	0.1 ± 0.02	NS
	0.04 (A)	0.03 (A)	0.04 (A)	
Zinc	11 ± 0.5	13 ± 1	8 ± 1	9 (0.009)
	11 (A)	12 (A)	8 (B)	

Means with the same letter are not significantly different using the Duncan Multiple Range Test. Results are in ppm (dry weight). NS: not significant.

blood from all of the snakes due to the difficulty of taking caudal blood; this accounts for the slightly difference size measurements for the snakes for which we had muscle and blood tissue samples.

There were some significant differences among the species with respect to levels of 14 elements (out of 18) in muscle (Table I), leading to a rejection of hypothesis 1. For muscle (tail tissue), cottonmouths had significantly higher levels of aluminum, arsenic, cobalt, copper, iron, mercury and vanadium than banded water snakes, and some of these were significantly higher than in brown water snakes. Brown water snakes had significantly higher levels of strontium than the other two species, and significantly higher levels of lead than banded water snakes. Banded water snakes had significantly higher levels of barium than the other species. Overall, of the 18 elements examined, cottonmouths exhibited the highest geometric means for 12 elements (whether they were significant or not).

There were fewer significant interspecific differences for blood (6 of 18 elements, Table I). Cottonmouths had significantly higher levels of barium in the blood than the other species, and higher levels of copper than brown water snake. Brown water snakes had higher levels of mercury than cottonmouths, and higher levels of selenium than banded water snakes. Both water snakes had higher levels of zinc than cottonmouths in the blood.

Thus, even for snakes living in the same environments, captured using the same bait in aquatic environments, there were significant interspecific differences in element levels.

3.2. EXPOSED AND REFERENCE SITE COMPARISONS

There were few significant differences in element levels for blood between Tim's Branch and the reference site for banded (nickel, uranium) and brown (aluminum, cobalt, chromium) water snakes, and none for cottonmouth (Table II).

There were more significant differences for muscle tissue (Table II). Arsenic and manganese were significantly higher in Tim's Branch compared to the reference site for all three species, and nickel and uranium were significantly higher for banded watersnake and cottonmouth, the larger species.

3.3. ELEMENTAL CORRELATIONS

There were relatively high significant correlations among elements for muscle of banded water snakes, the species with the largest sample sizes (Table III). All correlations were positive, except for mercury with manganese, and mercury with zinc. There were fewer significant correlations for the other two species, although they were equally high. These data indicate that, in general, individuals with high exposure of one element were exposed to high levels of other elements.

4. Discussion

4.1. INTERSPECIFIC COMPARISONS

The three species of aquatic snakes examined in this study are closely related, and have similar diets. Water snakes consume primarily fish and amphibians, but also insects and small mammals (Smith, 1961), and some water snakes can capture fish that are 18–20 cm long (Lagler and Salayer, 1945). They often specialize on bottom-dwelling fish (Raney and Roecker, 1947). Conant and Collins (1998) list the diet of cottonmouths as fish, frogs, salamanders, lizards, small turtles, baby alligators, birds and small mammals, and Wharton (1966) noted that they also obtain food by scavenging. All the species examined are not limited to an aquatic diet, but also take some terrestrial prey (Conant and Collins, 1998) and the water snakes on SRS eat a variety of prey, including sunfish (*Lepomis marginatus*), olden shiners (*Notemigonus crysoleucas*), red-fin pickerel *(Exox americanus*), pirate perch (*Aphredoderus sayanus*), green frog (*Rana clamitans*) and the southern toad (*Bufo terrestris*, W. Gibbons, personal communication). Cottonmouths may rely on a wider selection of prey, as well as eating snakes and rodents, than the water snakes (Clark *et al.*, 2000). Information on diets thus suggests that cottonmouths should exhibit the highest levels of elements, particularly contaminants, because they are heavier and eat a wider selection of prey (including other snakes which may accumulate contaminants).

In this study, where there were significant interspecific differences, cottonmouths more often had the highest levels than the other species. This was not always

TABLE II

Mean and standard error of metals in banded water snakes, brown water snakes, and cottonmouth snakes at Tim's Branch (Savannah River Site, Georgia) and the control site

	Reference	Tims	$X^2(p)$
Banded			
Snout-vent length (cm)	52 ± 4	52 ± 2	NS
Mass (g)	136 ± 33	140 ± 23	NS
Tail			
Sample size (n)	12	35	
Arsenic	0.2 ± 0.03	0.4 ± 0.04	11 (0.0009)
	0.2 (A)	0.4 (A)	
Cobalt	0.2 ± 0.01	0.3 ± 0.02	5 (0.04)
	0.2 (A)	0.2 (A)	
Iron	469 ± 33	731 ± 94	3 (0.09)
	458 (A)	514 (A)	
Mercury	0.9 ± 0.1	0.5 ± 0.05	8 (0.004)
	0.8 (A)	0.5 (B)	
Manganese	34 ± 7	77 ± 7	10 (0.001)
	29 (B)	47 (A)	
Nickel	3 ± 0	6 ± 1	3 (0.06)
	3 (B)	4 (A)	
Strontium	109 ± 8	89 ± 5	3 (0.09)
	106 (A)	60 (B)	
Uranium	0.05 ± 0.02	1 ± 0.2	23 (0.0001)
	0.02 (B)	0.7 (A)	
Blood			
Sample Size	9	25	
Nickel	0.09 ± 0.04	0.2 ± 0.03	10 (0.001)
Uranium	0.01 ± 0.00	0.05 ± 0.01	5 (0.03)
Cottonmouth	Reference	Tims	$X^2(p)$
Organ			
sv	56 ± 5	58 ± 6	NS
Mass	266 ± 46	289 ± 57	NS
Tail			
Sample size (n)	6	7	
Arsenic	0.4 ± 0.1	0.9 ± 0.1	7 (0.01)
	0.4 (B)	0.9 (A)	
Chromium	0.5 ± 0.1	2 ± 1	5 (0.02)
	0.4 (A)	1 (A)	

(*Continued on next page*)

TABLE II
(*Continued*)

	Reference	Tims	$X^2(p)$
Iron	811 ± 76	2141 ± 374	3.4 (0.06)
	793 (B)	1865 (A)	
Manganese	5 ± 0	32 ± 6	9 (0.03)
	5 (B)	28 (A)	
Nickel	3 ± 1	8 ± 2	7 (0.01)
	2 (B)	7 (A)	
Rubidium	22 ± 3	31 ± 4	4 (0.05)
	21 (A)	30 (A)	
Uranium	0.07 ± 0.02	2 ± 1	8 (0.004)
	0.05 (B)	1 (A)	
Brown	Reference	Tims	$X^2(p)$
Organ			
Snout-vent length (cm)	71 ± 9	63 ± 3	NS
Mass (g)	373 ± 15	192 ± 22	NS
Tail			
Sample size (*n*)	3	15	
Arsenic	0.4 ± 0.1	0.7 ± 0.1	3 (0.07)
	0.4 (A)	0.6 (A)	
Barium	48 ± 3	25 ± 3	4 (0.06)
	47 (A)	22 (B)	
Cobalt	0.30 ± 0.07	0.2 ± 0.02	4 (0.04)
	0.3 (A)	0.2 (B)	
Cesium	0.3 ± 0.04	0.1 ± 0.02	6 (0.02)
	0.3 (A)	0.1 (B)	
Iron	501 ± 34	2053 ± 403	5 (0.02)
	498 (A)	1464 (A)	
Manganese	49 ± 15	96 ± 10	4 (0.04)
	44 (A)	83 (A)	
Blood			
Sample size	2	7	
Aluminum	0.6 ± 0.3	2 ± 0.6	3 (0.08)
Cobalt	0.2 ± 0.07	0.05 ± 0.01	4 (0.04)
Chromium	0.02 ± 0.01	0.1 ± 0.02	4 (0.04)

Results are in ppm (dry weight).

the case, however, as banded water snakes (the smallest species) had higher levels of barium than the other species. However, barium was not an introduced contaminant into the Tim's Branch system. It thus seems that there are interspecific differences in uptake and bioaccumulation among the species that bears examination.

TABLE III
Significiant intermetal correlation (Kendall tau) of banded water snakes (tail, $n = 47$) collected from South Carolina

	r	p
Aluminun with		
Arsenic	0.23	0.03
Cobalt	0.32	0.002
Copper	0.35	0.0006
Iron	0.49	0.0001
Lead	0.36	0.0004
Manganese	0.3	0.002
Nickel	0.17	0.09
Selenium	0.21	0.04
Uranium	0.36	0.0004
Vanadium	0.6	0.0001
Zinc	0.39	0.0001
Arsenic with		
Cobalt	0.39	0.0002
Copper	0.3	0.003
Iron	0.39	0.0002
Lead	0.2	0.06
Manganese	0.3	0.004
Nickel	0.33	0.001
Selenium	0.3	0.005
Rubidium	0.21	0.04
Uranium	0.31	0.003
Zinc	0.27	0.01
Barium with		
Cobalt	0.25	0.02
Copper	0.18	0.07
Manganese	0.35	0.0005
Uranium	0.17	0.08
Vanadium	0.29	0.004
Zinc	0.18	0.07
Cobalt with		
Chromium	0.4	0.0001
Copper	0.43	0.0001
Iron	0.33	0.003
Lead	0.2	0.05
Manganese	0.47	0.0001

(*Continued on next page*)

TABLE III
(*Continued*)

	r	*p*
Nickel	0.43	0.0001
Selenium	0.43	0.0001
Rubidium	0.34	0.0007
Uranium	0.27	0.007
Vanadium	0.18	0.07
Zinc	0.37	0.0003
Chromium with		
Copper	0.027	0.008
Lead	0.2	0.05
Nickel	0.26	0.01
Selenium	0.37	0.0003
Rubidium	0.35	0.0006
Zinc	0.19	0.06
Cesium with		
Mercury	0.34	0.0008
Selenium	0.29	0.005
Rubidium	0.48	0.0001
Strontium	0.28	0.005
Copper with		
Iron	0.32	0.002
Lead	0.39	0.0002
Manganese	0.35	0.0005
Nickel	0.37	0.0003
Selenium	0.36	0.0003
Rubidium	0.24	0.02
Uranium	0.3	0.003
Vanadium	0.23	0.02
Zinc	0.61	0.0001
Iron with		
Lead	0.29	0.005
Manganese	0.31	0.002
Uranium	0.29	0.005
Vanadium	0.39	0.0001
Zinc	0.35	0.0007
Lead with		
Nickel	0.23	0.03
Selenium	0.23	0.03

(*Continued on next page*)

TABLE III
(*Continued*)

	r	*p*
Vanadium	0.18	0.09
Zinc	0.47	0.0001
Manganese with		
Mercury	−0.23	0.02
Nickel	0.36	0.0005
Rubidium	0.2	0.04
Uranium	0.48	0.0001
Vanadium	0.26	0.01
Zinc	0.36	0.0003
Mercury with		
Selenium	0.3	0.003
Strontium	0.34	0.0008
Uranium	−0.32	0.002
Nickel with		
Selenium	0.22	0.03
Rubidium	0.32	0.002
Strontium	0.19	0.06
Uranium	0.33	0.001
Zinc	0.46	0.0001
Selenium with		
Rubidium	0.23	0.02
Strontium	0.34	0.0007
Zinc	0.25	0.01
Rubidium with		
Zinc	0.23	0.02
Uranium with		
Vanadium	0.28	0.005
Zinc	0.35	0.0005
Vanadium with		
Zinc	0.23	0.02

4.2. GEOGRAPHICAL COMPARISONS

Element levels in water snakes and cottonmouth have been examined for a limited number of elements in a small number of places. Water snakes (*Nerodia* spp.) were collected from the upper and lower reaches of the Apalachicola River, Florida in 1978, and whole-body composite samples were analyzed for arsenic, cadmium, lead, mercury, and selenium as part of a contaminant study of Apalachicola River aquatic biota (Winger *et al.*, 1984). Average levels for the water snakes in their sample were 60 ppb for arsenic, 20 ppb for cadmium, 480 ppb for lead, 290 ppb for mercury, and 440 ppb for selenium (wet weights). For the snakes at SRS: (1) the levels of arsenic were about four times higher overall, and even in the reference site they were higher (our dry weights converted by dividing by 3), (2) lead levels were two to four times higher, (3) mercury levels were about the same, and (4) selenium levels were similar for tail tissue.

Niethammer *et al.* (1985) analyzed cadmium and lead levels in carcasses (minus head, skin, and gastrointestinal tract) of northern water snakes collected in 1981–1982 from the Big River and Black River drainages in two lead mining districts of southeastern Missouri. Cadmium levels in water snakes were usually below the limits of detection (0.1 ppm, wet weight). Mean lead concentrations in snakes collected from the downstream Big River sites (6070 and 7520 ppb wet weight, respectively) were significantly higher than those from the upstream, uncontaminated Big River site (190 ppb, wet weight) and the Black River sites (1210, 970, and 150 ppb wet weight).

Clark *et al.* (2000), working in Texas, reported mean levels of copper to be 899 ppb (all wet weights) for cottonmouths, and 586–648 ppb for diamondback water snakes; mean mercury was 13.5 ppb for cotton mouths, and 61–146 ppb for watersnakes; mean selenium was 277 ppb for cottonmouths and 318–352 ppb for water snakes; mean zinc was 8880 ppb for cottonmouths, and 13,600–13,900 ppb for water snakes. The snakes in our SRS study has slightly higher levels of copper and selenium, higher levels of mercury, and much higher levels of zinc. Zinc is one of the elements of concern for Tim's Branch at SRS (Punshon *et al.*, 2004).

Hopkins *et al.* (1999) analyzed arsenic, cadmium, chromium, copper, and selenium concentrations in livers of banded water snakes from a coal-burning power plant on the Savannah River Site and a nearby reference site (five snakes from each site). Levels of contaminants from the power plant site averaged about 135,000 ppb for arsenic, 140,000 ppb for selenium, 500 ppb for cadmium, and 2000 ppb for chromium (dry weight), all predictably higher than snakes from this study since these elements were not contaminants of concern. Levels for the livers from the reference site were lower (230 ppb arsenic, 3620 ppb for selenium, 120 ppb for cadmium, 800 ppb for chromium), were more similar to those we found for tail tissue.

Overall, these data indicate that the elemental levels in the tissues of water snakes from SRS reported in this study are higher than for many places (Winger *et al.*, 1984; Clark *et al.*, 2000). Unfortunately, there are insufficient data from other studies on the other elements, eliminating the possibility of direct comparisons.

4.3. TIM'S BRANCH AND REFERENCE SITES

In general, levels of most elements were higher in the tissues of snakes collected in Tim's Branch, as might be expected. However, there were some interesting exceptions where levels were higher in tissues from the reference site compared to Tim's Branch: (1) mercury and strontium were significantly higher in banded water snakes, (2) barium, cobalt and cesium were significantly higher for brown water snakes. However, levels in cottonmouths were always higher (or not different) in Tim's Branch compared to the reference site. We cannot account for these differences since the reference site was chosen with the expectation that the contaminants of concern would be low. However, other wildlife studies have reported that trace elements, although at background levels, were higher in the Upper Three Runs watershed than other SRS reference sites. This could be due in part to specific biogeochemical properties of this large riparian system (Gaines *et al.*, 2002; Lord *et al.*, 2002).

The elements of concern for Tim's Branch are uranium and nickel, as well as copper and zinc and other heavy metals (Punshon *et al.*, 2003). Although nickel and uranium were significantly higher in banded water snakes and cottonmouth muscle tissue collected from Tim's Branch, this was not the case for brown watersnakes. This, however, may be due to relatively small sample sizes. Surprisingly, copper and zinc levels were not significantly higher in the muscle or blood tissues from any of the three species of snakes examined in this study. This suggests that the sources of the metals at the reference site should be examined.

Further, Murray (2003) has shown that availability of metals may also have been influenced by the relative trophic positions of primary and secondary consumers, although the dynamic food web in Tim's Branch has still not been entirely defined. Uranium and nickel accumulates within the tissue of the water snake, however plant and soil concentrations were 3–4 orders of magnitude greater than the body burdens of the snake collected. Transfer factors from soil to snake could not be estimated without data to quantify the amphibians diet, which may have linked soil concentration of uranium and nickel to the snakes. Further, this study showed that the presence of uranium in muscle tissue correlated with DNA double-strand breakage re-emphazing that although uranium and other potentially toxic metals may not biomagnify or bioaccumalate in high levels, they must be monitored from a hierarchal ecosystem approach rather than at the compartment level.

4.4. SNAKES AS BIOINDICATORS

Snakes are useful bioindicators in both terrestrial and aquatic environments because they are often at the top of the food chain (Andreadis, 1998; Campbell and Campbell, 2001; Burger *et al.*, in press). Moreover, some snakes such as water snakes, are sufficiently common that collection for scientific purposes does not pose any risk of population effects on the snakes themselves. Blood and tail muscle are two of the tissues often used. In this study, there were interspecific differences in 14 elements for muscle, and for 6 of 18 elements for blood. Since the deposition of elements into body tissue, such as blood, may have a quicker turnover time, especially for trace elements that have not reached accumulation thresholds, tail snips may be a better indicator of ecosystem health for a longer temporal scale. That is, while it is useful to obtain a measure of recent exposure, for long term biomonitoring, more robust measures of a longer exposure period may be more important for understanding potential risk to the snake populations themselves. The snakes in this study had relatively high levels of some elements compared to aquatic snakes from elsewhere, suggesting that contaminant levels on Tim's Branch need to be closely monitored.

There are two issues that bear mention: (1) lack of comparative data on elemental levels in snakes, particularly aquatic snakes, and (2) use of wet and dry weight in the literature. There are no reports in the literature for levels of many of the elements we analyzed. and where they exist, they are not for blood and muscle. An important methodological issue is that some authors provide results in wet weight, while others report dry weights, making direct comparisons difficult. While there is generally three times the levels in dry weight as wet weight (as a function of the percent water in the sample), this is not uniformly true for different tissues and for different species. We feel there is a great need for standardization in methodology for examining elemental levels in biota.

Finally, it is critical to bear in mind that no one indicator is sufficient to evaluate any given contaminated site. Instead, there should be a suite of indicators that provides information at a range of different trophic levels, and in different habitats. We suggest that for southern hardwood forests, swamps, and aquatic habitats, snakes can be used as bioindicators, in concert with raccoons (*Procyon lotor*), doves, and fish (Kennamer *et al.*, 1998; Burger *et al.*, 2000, 2001a,b, 2002; Gaines *et al.*, 2000a,b; Lord *et al.*, 2002). This suite of indicators includes aquatic and terrestrial organisms, as well as species at different trophic levels.

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References

Andreadis, P. T.: 1998, 'Control of Food Intake and Expression of Hunger in the Northern Water Snake, *Nerodia sipedon* (L.)', *Ph.D. Dissertation*, University of Tennessee, Knoxville, Tennessee, 186 p.

Barron, M. G.: 1995, 'Bioaccumulation and Bioconcentration in Aquatic Organisms', in: D. J. Hoffman, B. A. Rattner, G. A. Burton Jr., and J. Cairns Jr. (eds), *Handbook of Ecotoxicology*, Lewis Publisher, Boca Raton, Florida, pp. 652–666.

Bauerle, B.: 1975, 'The use of snakes as pollution indicator species', *Copeia* 1975; 367–368.

Bertsch, P. M., Sowder, A. G., Punshon, T., Gaines, K. F., Adriano, D. C. and Brisbin, I. L. Jr.: 2000, *Environmental Availability, Bioavailability, and Trophic Transfer of Sediment Bound Heavy Metals in a Southeastern Riparian/Wetland System*, SREL/DOE Report, Aiken, SC.

Bishop, C. A. and Rouse, J. D.: 2000, 'Chlorinated hydrocarbon concentrations in plasma of Lake Erie water snake (*Nerodia sipedon insularum*) and northern water snake (*Nerodia sipedon sipedon*) from the Great Lakes Basin in 1998', *Arch. Environ. Contam. Toxicol.* **39**, 500–505.

Burger, J.: 2002, 'Food chain differences affect heavy metals in bird eggs in Barnegat Bay, New Jersey', *Environ. Res.* **90**, 33–39.

Burger, J., Lord, C. G., McGrath, L., Gaines, K. F., Brisbin, I. L., Jr., Gochfeld, M. and Yurkow, E. J.: 2000, 'Metals and Metallothionein in the liver of raccoons: Utility for environmental assessment and monitoring', *J. Toxicol. Environ. Health* **60**, 243–261.

Burger, J., Gaines, K. F., Boring, S., Stephens, W. L. Jr., Snodgrass, J. and Gochfeld, M.: 2001a, 'Mercury and selenium in fish from the Savannah River: Species, trophic level, and locational differences', *Environ. Res.* **87**, 108–118.

Burger, J., Gaines, K. F., Stephens, W. L. Jr., Boring, C. S., Brisbin, I. L. Jr., Snodgrass, J., Peles, J., Bryan, L., Smith, M. J. and Gochfeld, M.: 2001b, 'Radiocesium in fish from the Savannah River and Steel Creek: Potential food chain exposure to the public', *Riak Anal.* **21**, 545–559.

Burger, J., Gaines, K. F., Boring, C. S., Stephens, W. L. Jr., Snodgrass, J., Dixon, C., McMahon, M., Shukla, S., Shukla, T. and Gochfeld, M.: 2002, 'Metal levels in fish from the Savannah River: Potential hazards to fish and other receptors', *Environ. Res.* **89**, 85–87.

Burger, J., Campbell, R. S., Campbell, T. S., Shukla, T., Jeitner, C., and Gochfeld, M.: in press, 'The use of blood and skin as non-destructive indicators of heavy metal contamination in northern water snakes (*Nerodia sipedon*)', *Environ. Monit. Assess.*

Campbell, K. R. and Campbell, T. S.: 2001, 'The accumulation and effects of environmental contaminants on snakes: A review', *Environ. Monitor. Assess.* **70**, 253–301.

Clark, D. R., Jr., Bickham, J. W., Baker, D. L. and Cowman, D. F.: 2000, 'Environmental contaminants in Texas, USA, wetland reptiles: Evaluation using blood samples', *Environ. Toxicol. Chem.* **19**, 2259–2265.

Conant, R. and Collins, J. T.:1998, *A Field Guide to Reptiles and Amphibians of Eastern and Central North America*, Houghton Mifflin Co, Boston, MA.

Ernst, C. H. and Barbour, R. W.: 1989, *Snakes of Eastern North America*, George Mason University Press, Fairfax, VA.

Fitzgerald, W. F.: 1989, 'Atmospheric and Oceanic Cycling of Mercury', in: J. P. Riley and R. Chester (eds), *Chemical Oceanography*, Academic Press, New York, pp. 151–186.

Ford, W. M. and Hill, E. P.: 1991, 'Organochlorine pesticides in soil sediments and aquatic animals in Upper Steel Bayou Watershed of Mississippi', *Arch. Environ. Contam. Toxicol.* **20**, 161–167.

Gaines, K. F., Lord, C. G., Brisbin, I. L. Jr., Boring, C. S., Gochfeld, M. and Burger, J.: 2000a, 'Radiocesium in raccoons: Population differences and potential human risks', *J. Wildl. Manage.* **64**, 199–208.

Gaines, K. F., Lord, C. G., Boring, C. S., Brisbin, I. L. Jr., Gochfeld, M. and Burger, J.: 2000b, 'Raccoons as potential vectors of radionuclide contaminantion to human food chains from a nuclear industrial site', *J. Wildl. Manage.* **64**, 188–208.

Gaines, K. F., Romanek, C. S., Boring, C. S., Lord, C. G., Burger, J. and Gochfeld, M.: 2002, 'Using raccoons as an indicator species for metal accumulation across trophic levels-a stable isotope approach', *J. Wildl. Manage.* **66**, 808–818.

Hopkins, W. A., Rowe, C. L. and Congdon, J. D.:1999, 'Elevated trace element concentrations and standard metabolic rate in banded water snake (*Nerodia fasciata*) exposed to coal combustion wastes', *Environ. Toxicol. Chem.* **18**, 1258–1263.

Hopkins, W. A., Roe, J. H., Snodgrass, J. W., Jackson, B. P., Kling, D. E., Rowe, C. L. and Congdon, J. D.: 2001, 'Nondestructive indices of trace elements exposure in squamate reptiles', *Environ. Pollut.* **115**, 1–7.

Kennamer, R. A., Brisbin, I. L. Jr., McCreedy, C. D. and Burger, J.: 1998, 'Radiocesium in Mourning Doves foraging on the exposed lakebed of a contaminated reactor-cooling reservoir: Risk to human consumers and temporal effects of the drawdown', *J. Wildl. Manage.* **62**, 497–508.

Lagler, K. F. and Salayer, J. C., II: 1945, 'Food and habits of the common water snake, Natrix sipedon, in Michigan', *Michigan Acad. Sci. Arts Lett.* **31**, 169–180.

Linthurst, R. A., Bourdeau, P. and Tardiff, R. G.: 1995, *Methods to Assess the Effects of Chemicals in Ecosystems*, John Wiley and Sons, Chichester, UK.

Lord, C. G., Gaines, K. F., Boring, C. S., Brisbin, I. L., Gochfeld, M., and Burger, J.: 2002, 'Raccoon (*Procyon lotor*) as a bioindicator of mercury contamination at the U.S. Department of Energy's Savannah River Site', *Arch. Environ. Contam. Toxicol.* **43**, 356–363.

Mailman, R. B.: 1980, 'Heavy metals', in: J. J. Perry (eds), *Environmental Toxicology*, Elsevier, New York, pp. 34–43.

Murray, S.: 2003, 'Can Exposure Models Predict Endpoint Effects: A Case Study Using Water Snakes', *Ph.D. Dissertation*, Rutgers University, Piscataway, New Jersey.

Pickett, J. B.: 1990, Heavy Metal Contamination in Tim's Branch Sediments, *Report of Westinghouse Savannah River Company*, Aiken, South Carolina.

Punshon, T., Bertsch, P. M., Lanzirotti, A., McLeod, K. W. and Burger, J.: 2003, 'Geochemical signature of contaminated sediment remobilization revealed by spatially resolved x-ray micoranalysis of annual rings of Salix nigra', *Environ. Sci. Technol.* **37**, 1766–1774.

Punshon, T., Jackson, B. P., Bertsch, P. M. and Burger, J.: 2004, 'Mass loading of nickel and uranium on plant surfaces: Application of laser ablation-ICP-MS', *J. Environ. Monit.* **6**, 153–159.

Raney, E. C. and Roecker, R. M.: 1947, 'Food and growth of two species of watersnakes from western New York', *Copeia* **1947**, 171–174.

SAS: 1995, *SAS User's Guide*, Statistical Analysis Institute, Cary, NC.

Smith, P. W.: 1961, *The Amphibians and Reptiles of Illinois*, Illinois Natural History Survey Bulletin, Vol. 28.

Wharton, C. H.: 1966, 'Reproduction and growth in the cottonmouths, Agkistrodon piscivorus Lacepede, of Cedar Keys, Florida', *Copeia* **1966**, 149–161.