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Arsenic, Cadmium, Chromium, Lead, Mercury and Selenium Concentrations in Pine Snakes (*Pituophis melanoleucus*) from the New Jersey Pine Barrens

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Abstract Top trophic level predators are at risk from bioaccumulation of heavy metals from their prey. Using nondestructively collected tissues as a method of assessing metal concentrations in snakes is useful for populations that are threatened or declining. This paper reports concentrations of arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), and selenium (Se) in tissues of Northern pine snakes (*Pituophis melanoleucus*) from the New Jersey Pine Barrens, a relatively pristine, undisturbed habitat. We also determined if skin is an appropriate indicator of internal concentrations and identified the factors (tissue, year of collection, length, sex) that might explain variations in metal concentrations. Because they can grow to 2-m long and live for 25 years, we suggest that these snakes might accumulate heavy metals. Multiple regression models were significant, explaining 16% (lead) to 61% (mercury) of variation by tissue type. For mercury and chromium, size also was significant. The highest concentrations were in liver and kidney for all metals, except chromium and lead. Mercury concentrations in tissues were within the range reported for other snakes and were below effects concentrations in reptiles. The concentrations in skin were correlated with all internal tissues for mercury and for all internal tissues except heart for cadmium. These data show that shed skin can be used as an indicator of metals in pine snakes and that, at present, concentrations of heavy metals in this population are within the range of those found in other snake species from uncontaminated sites.

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Governmental agencies, public policy makers, eco-toxicologists, conservationists, managers, and the public are interested in concentrations of contaminants in the environment that could have adverse effects on wildlife themselves, or for the organisms that consume them. Environmental contamination is a global problem because of the potential adverse effects of chemicals on humans and the environment. Contaminants enter the food chain through natural erosion, biogeochemical processes, and industrial or other anthropogenic sources. While concentrations of chemicals often are examined in biota living in aquatic and marine systems (Furness and Rainbow 1990; Burger and Gochfeld 2002, 2016), there are fewer studies in inland forested areas. Yet, chemicals are atmospherically transported all over the world, including to relatively isolated, inland regions (Fitzgerald 1989; Houghton et al. 1992; Hammerschmidt and Fitzgerald 2006). Airborne metals are dispersed on land and water, and enter both terrestrial and aquatic food chains. Plants take up metals into their foliage and fruits that are consumed by invertebrates that are eaten by rodents, which form the prey base for many predators, including snakes. Predators that are at the top of their food web are more vulnerable, because they typically accumulate higher concentrations of metals than species at lower trophic levels (Niethammer et al. 1985; Hopkins et al. 1999, 2001; Burger and Gochfeld 2016).

Although there has been considerable attention devoted to determining the concentrations and adverse effects of metals in birds, mammals, and fish, and even for some groups of reptiles, there has been relatively little attention to metal concentrations in snakes (Delany et al. 1988; Campbell and Campbell 2000, 2001; Marquez-Ferrando et al. 2009) or to the toxic effects of contaminants on behavior and reproduction of snakes (Burger 2006; Schneider et al. 2013). Snakes have received little attention, because they are solitary and secretive, sufficient numbers may not be available for analysis, and because use of reptiles is not part of the usual protocol of the Environmental Protection Agency. Metals have been studied in lizards (Marquez-Ferrando et al. 2009; Salice et al. 2009), alligators (Camus et al. 1998; Burger et al. 2000), and turtles (Stoneburner et al. 1980; Bishop et al. 1991, 1995; Caurant et al. 1999; Burger 2002; Day et al. 2005; Kampalath et al. 2006; Gardner et al. 2006; Lam et al. 2006; Talavera-Saenz et al. 2007; Bergeron et al. 2007).

Northern pine snakes are large constrictors that reach the northern limit of their range in the New Jersey Pine Barrens. They are among the top-level predators in the region that can grow to more than 2-m long (Burger and Zappalorti, unpub. data). The genus *Pituophis* has four species: Pine Snake (*P. melanoleucus*), Bull and Gopher Snakes (*P. catenifer and P. sayi*), and Louisiana pine snake (*P. ruthveni*), which is a candidate for federally threatened status. *Pituophus melanoleucus* has three subspecies: the Florida black pine snake (*P. m. mugitus*), the black pine (*P. m. loding*), which is federally threatened (Federal Register 2015), and the Northern pine snake (*P. m. melanoleucus*), which is threatened in New Jersey. The New Jersey population of Northern pine snakes is isolated from other populations living to the south by several hundred kilometers (Burger and Zappalorti 2011, 2016). This species is declining in many parts of its range and is common nowhere.

Metal concentrations have been examined in snakes near contaminated sites at the Savannah River site (South Carolina, USA, Burger et al. 2006), at Mobile-Tensaw River Delta (Alabama, USA, Albrecht et al. 2007), and at South River (Virginia, USA, Drewett et al. 2013), as well at uncontaminated sites in the Pine Barrens (New Jersey, USA, Burger 1992), the Raritan Canal (New Jersey, USA, Burger et al. 2007), and the northeast coast of the Persian Gulf (Iran, Rezaie-Atagholipour et al. 2012; Sereshk and Bakhtiari 2015). Metal concentrations have been examined in whole bodies of snakes (Albrecht et al. 2007), in tail muscle tissue (Drewett et al. 2013), and in shed skins (Jones and Holladay 2006; Wylie et al. 2009). Although skin of snakes is regularly moulted, the present study shows that metals are sequestered in the skin, making them usable as a bioindicator, much as human hair is used as an indicator of metal exposure (Burger 1992).

The present study examined the concentrations of arsenic, cadmium, chromium, lead, mercury, and selenium in 20 pine snakes from the Pine Barrens of New Jersey (Fig. 1), ranging in size from 40 cm (hatchlings) to 159 cm (adults) snout–vent length. These were found dead (2010–2016), mainly killed by being hit by cars on roads. Thus, these snakes did not die of natural causes. Blood samples were obtained from an additional 29 live pine snakes between 2013 and 2015. Objectives were to: (1) examine concentrations in blood and other tissues as a function of size and sex, (2) determine if concentrations of metals are intercorrelated, and (3) determine if the levels are high enough to suggest adverse effects and cause for concern. Pine snakes (*Pituophis melanoleucus*) are emblematic of New Jersey's Pine Barrens habitats (Burger et al. 2013). Pine snakes might be useful indicators of contamination for the Pine Barrens of New Jersey, because they are long-lived and top-trophic level predators (Burger et al. 2013; Burger and Zappalorti 2016). It is rare that a sample of large snakes, such as pine snakes, can be found dead, allowing for examining different tissues in the same individuals. The Pine Barrens are relatively pristine compared with other urban areas of the Northeast, in that they are protected as a national reserve, and have little industry and no point sources of pollution, which suggests that concentrations of metals will be low relative to other snakes from urban areas of the Northeast with diverse industries.

Materials and Methods

Pine snakes in New Jersey dig their own nests and modify and dig their own hibernacula (Burger and Zappalorti 1992; Burger 2006). Both pine snakes and gopher snakes are most active from early April to late October (Burger et al. 1988; Rodriguez-Robles 2003). Pine snakes nest in late June to early July, and incubation temperatures affect behavior and survival of hatchlings (Burger and Gochfeld 1985; Burger et al. 1987; Burger and Zappalorti 1988a, b; Burger 1989a, 1991a, 1998a, b). The hatchlings emerge from the nests and follow chemical trails to find hibernacula and to avoid predators (Burger 1989b, 1990, 1991b; Burger et al. 1991). Northern pine snakes are vulnerable to the usual threats of habitat loss, insufficient food supplies, predators, inclement weather, and finding secure hibernation sites (especially hatchlings), but mainly they suffer from killing, mortality on roads, and poaching (Schwartz and Golden 2002; Sherwood et al. 2002; Golden et al. 2009; Burger and Zappalorti 2011, 2016; Smith et al. 2015). Examining whether concentrations of heavy metals

Fig. 1 Map of New Jersey, indicating the location of the New Jersey Pine Barrens, where pine snakes were collected for metal analysis, mainly from roads

are sufficiently high to cause adverse effects that might impact population levels needs to be determined before metals can be ruled out as a potential cause of population declines.

In this study, we examined metal concentrations in blood of 29 pine snakes (collected from live snakes during hibernation studies in 2012–2013), and tissues from 20 different pine snakes killed by vehicles, predators, fires, or freezing from 2006 to 2015). Blood was taken from snakes that lived in the interior of the Pine Barrens, mainly from snakes living in state forests where there are no houses, no paved roads, and no farming. These snakes were sampled and released, and only blood was collected from these individuals. The 20 snakes that were found dead also were from the Pine Barrens but were from areas with paved roads, houses, and light industry, as well as protected pine forests. The same tissues were collected from each of the 20 snakes and included skin, muscle, liver, kidney, and heart, except in rare cases where these were unavailable (e.g., due to damage). Because the species is threatened in New Jersey, we did not kill snakes for analysis, nor did we take tail clips. Concentrations in the liver, kidney and heart are indicative of internal exposure that might affect the health and well-being of the snakes themselves, concentrations in muscle reflect exposure of predators that might eat the pine snakes, and skin was examined as a potential indicator of internal exposure.

Metal analyses were conducted at the Environmental and Occupational Health Sciences Institute of Rutgers University in Piscataway, New Jersey. Using Atomic Spectrophotometer with Zeeman correction (Burger 2002; Burger et al. 2006, 2007). For quality assurance, all analyses reported here were done in 2016 (to avoid any methodological issues if tissues were analyzed in different years). All results are reported as ppb (ng/g) on a wet weight basis. All laboratory equipment and containers were washed in 10% nitric acid solution and rinsed with deionized water before each use. A 0.2-g (wet weight) sample of tissue was digested in 4 ml of 70% Fisher TraceMetal nitric acid and 2 ml of deionized water in a microwave (MDS 2000 CEM), using a digestion protocol of three stages of 10 min each under 50, 100, 150 pounds/in^2 (3.5, 7, and 10.6 kg/cm^2) at 70% power. Digested samples were subsequently diluted to 10 ml with deionized water. Detection limits were: 0.02 ppb for arsenic, 0.01 ppb for cadmium, 0.08 ppb for chromium, 0.15 ppb for lead, 0.2 ppb for mercury, and 0.7 ppb for selenium. All specimens were analyzed in batches with known standards, calibration standards, and spiked specimens. Recoveries ranged from 88 to 102%. The coefficient of variation on replicate, spiked samples ranged up to 10%.

Multiple regression models were used to determine the best models explaining variations in metal concentrations as a function of tissue, year of capture, length, weight, and sex (SAS 2005). Kruskal–Wallis nonparametric analysis of variance was used to compare tissues for each metal. Kendall tau correlations were used to examine the relationships between skin concentrations and tissue concentrations, among organs for each metal, and between length and metal concentrations. A probability level of 0.05 was accepted as significance, but because of the sample size we present all values < 0.1 to allow the reader to assess the significance themselves.

Results

For all metals, there was substantial variation in metal concentrations among tissues ($p < 0.004$ for all metals). The multiple regression models explained 16% to 61% of the variation in metal concentrations, with tissue type entering all models (Table 1). Most models explained less than 50% of the variation. The model for variations in mercury concentrations explained 61% of the variation in terms of tissue type and snout–vent length. Year and weight might have entered the models significantly if there were larger sample sizes.

Although metal concentrations varied significantly by tissue for all metals (Table 2), the same tissue type did not always have the highest concentrations. Of the 20 snakes for which we had internal tissues (except blood), skin had the lowest concentrations of arsenic, cadmium, mercury, and selenium. Liver and kidney had the highest mean concentrations of arsenic, cadmium, mercury, and selenium.

Surprisingly, size as measured by snout–vent length, was negatively related to metal concentration for chromium in heart muscle ($\tau = -0.46$, $p = 0.02$) and skin ($\tau = -0.29$, $p < 0.07$). For mercury in blood the relationship was also negative ($\tau = -0.25$, $p < 0.04$), whereas for mercury in liver there was a significant positive relationship ($\tau = 0.30$, $p < 0.05$).

Interyear differences were significant only for chromium and lead in blood ($\tau = 0.38$, $p < 0.01$; $\tau = 0.40$, $p < 0.40$, $p < 0.009$, respectively), and for selenium in skin (τ -0.46, $p < 0.02$). Thus, in general, year was not a significant variable for many metal-tissue combinations.

Sex did not enter any of the models as a significant contributor to variability, and there was only one significant difference when means were compared (nonparametric ANOVA, SAS, 2005). Females had lower concentrations of mercury in the kidney than did males (Table 3).

Because some investigators use skin as an indicator of internal tissue concentrations, we examined the relationship between concentrations of metals in skin and concentrations in other tissues (Table 4). For mercury, the concentrations in skin were significantly correlated with all

Table 1 Multiple regression models on metal levels in pine snakes collected from New Jersey

	Arsenic	Cadmium	Chromium	Lead	Mercury	Selenium
Model						
F(*p*)	5.0 (<0.0001)	4.2 (0.0001)	5.7 (<0.0001)	2.2 (0.03)	17.0 (<0.0001)	10.4 (<0.0001)
df	9	9	9	9	9	9
r^2	0.31	0.27	0.34	0.16	0.61	0.48
Factors entering *F* (*p*)						
Tissue[a]	4.76 (0.0006)	6.3 (<0.0001)	3.9 (0.003)	2.7 (0.02)	26.4 (<0.0001)	15.7 (<0.0001)
Year[b]	NS	NS	NS	NS	3.0 (0.09)	11.9 (0.0008)
Snout–Vent length[c]	NS	NS	11.9 (0.0008)	NS	5.8 (0.02)	NS
Weight	NS	NS	NS	NS	3.3 (0.07)	NS
Sex[d]	NS	NS	NS	NS	NS	NS

NS not significant

[a] Tissue includes blood, muscle, kidney, heart, skin, and liver

[b] Years are from 2001 to 2016

[c] Size range from hatchling (43 cm) to adult (158 cm)

[d] Sample includes 13 females and 7 males

Table 2 Metal levels (ppb, ng/g wet weight) in pine snakes collected from New Jersey

Tissue	*n*	Arsenic Mean ± SE	Cadmium Mean ± SE	Chromium Mean ± SE	Mercury Mean ± SE	Lead Mean ± SE	Selenium Mean ± SE
Liver	17	156 ± 27.3	128 ± 30.2	45.0 ± 8.7	459 ± 78.1	140 ± 38.6	985 ± 141
Kidney	15	167 ± 53.2	95.1 ± 31.9	51.0 ± 19.6	120 ± 35.0	352 ± 129	1213 ± 240
Muscle	20	125 ± 31.3	27.2 ± 8.2	150 ± 21.8	75.8 ± 11.9	393 ± 131	217 ± 40.7
Skin	20	91.4 ± 21.7	19.8 ± 3.4	161 ± 46.0	41.5 ± 7.1	172 ± 57.0	182 ± 35.8
Heart	15	145 ± 47.3	51.0 ± 28.4	72.9 ± 32.1	41.3 ± 9.5	86.7 ± 23.3	305 ± 68.8
Blood	29	7.0 ± 3.3	4.3 ± 2.7	42.5 ± 7.8	26.9 ± 5.5	88.8 ± 15.1	502 ± 46.9
Kruskal–Wallis χ^2		42.3 (<0.0001)	46.6 (<0.0001)	32.3 (<0.0001)	62.0 (<0.0001)	17.5 (0.004)	44.0 (<0.0001)

Given are arithmetic means ± SE, and non-parametric oneway analysis of variance using the Kruskal–Wallis Chi-square (*p*). Blood samples were from different snakes than the other tissues, which were from the same individuals. All values are for total metal without speciation

internal tissues, and for cadmium they were significant for all tissues except the heart. Chromium concentrations in skin were significantly correlated in muscle and liver tissue, and selenium concentrations in skin were significantly correlated in liver and kidney. The concentrations of arsenic in skin were not correlated with internal tissue.

In this paper, we report concentrations as wet weights. However, in the literature some studies report wet weight and some report dry weight. Thus, we provide the wet to dry conversion factors for our data (Table 5).

Discussion

Tissue, Size and Sex Differences

There were significant differences among tissues types for all metals, although the differences were not great. Nearly all studies with reptiles find tissue differences (Campbell and Campbell 2001, 2002), which is not surprising given the structure and function of different tissues. There were few differences as a function of snout–vent length and sex, although mercury was significantly correlated with snout–vent length for liver, and negatively correlated in blood. The positive correlation of mercury in liver was expected as mercury is usually correlated with size and age in a variety of vertebrates, indicating biomagnification (Schneider et al. 2013; Burger and Gochfeld 2016). The negative correlation of mercury in blood with size was unexpected and bears further comment. Similarly, the negative correlation of snout–vent length with mercury concentrations in skin was unexpected. It may be that with increasing size and internal tissues, larger animals have greater compartments for disposition of mercury. Females had significantly lower

Table 3 Sex differences in metal levels (ppb, wet weight) (ng/g) of pine snakes

	Male $n = 6$	Female $n = 14$	χ^2 (p)
Snout–Vent length (cm)	112 ± 15.6	115 ± 9.8	NS
Weight (g)	797 ± 238.0	577 ± 103.0	NS
Skin			
Arsenic	94 ± 47	90 ± 25	NS
Cadmium	15 ± 5.2	22 ± 4.3	NS
Chromium	79 ± 18	196 ± 64	NS
Lead	103 ± 40	201 ± 79	NS
Mercury	29 ± 8.6	47 ± 9.3	NS
Selenium	207 ± 66	171 ± 44	NS
Muscle			
Arsenic	111 ± 44	131 ± 42	NS
Cadmium	26 ± 18	28 ± 9.4	NS
Chromium	136 ± 42	156 ± 26	NS
Lead	327 ± 177	421 ± 175	NS
Mercury	48 ± 11	87 ± 16	NS
Selenium	243 ± 68	205 ± 52	NS
Liver			
Arsenic	145 ± 44	162 ± 36	NS
Cadmium	125 ± 42	130 ± 42	NS
Chromium	57 ± 16	39 ± 10	NS
Lead	166 ± 73	126 ± 47	NS
Mercury	287 ± 89	553 ± 102	NS
Selenium	1197 ± 293	870 ± 147	NS
Kidney			
Arsenic	273 ± 143	115 ± 34	NS
Cadmium	103 ± 89	91 ± 24	NS
Chromium	100 ± 54	26 ± 7.3	NS
Lead	294 ± 167	380 ± 180	NS
Mercury	48 ± 12	156 ± 49	6.0 (0.01)
Selenium	980 ± 267	1329 ± 337	NS
	$n = 14$	$n = 15$	
Blood			
Snout–Vent length (cm)	127 ± 3.9	129 ± 2.9	NS
Weight (g)	930 ± 84	845 ± 52	NS
Arsenic	6.8 ± 4.0	7.1 ± 5.3	NS
Cadmium	6.0 ± 5.3	2.7 ± 1.8	NS
Chromium	48 ± 14	37 ± 7.8	NS
Lead	118 ± 27	62 ± 12	NS
Mercury	30 ± 10	24 ± 5.1	NS
Selenium	522 ± 64	483 ± 70	NS

Given are arithmetic means ± SE with Kruskal–Wallis Chi-square values and p values

concentrations of mercury in the kidney, compared with males, which might reflect the deposition of mercury in eggs.

Comparisons of Metal Concentrations with Other Snakes

Of the reptile studies on mercury concentrations, only 9% were on snakes, and there are many more studies with organic contaminants (Campbell and Campbell 2001; Schneider et al. 2013). There are few studies of concentrations of metals in snakes under uncontaminated conditions. However, Wylie et al. (2009) examined trace elements in Giant Garter Snakes (*Thamnophis gigas*) in California, and Albrecht et al. (2007) reported on metals in Ribbon Snakes (*Thamnophis sauritis*) in Alabama; levels were very low in these studies from uncontaminated sites. Most studies have used snakes as indicators of environmental pollution, comparing metal levels in snakes from a contaminated site with those living in a reference site (Hopkins et al. 1999; Burger et al. 2006, 2007), or comparing bioaccumulation by different snake species inhabiting a contaminated site (Drewett et al. 2013). In these studies, snakes from contaminated sites had higher concentrations than those from the reference sites (often twice the concentration or more). The concentrations from contaminated sites were also higher than those found in the present study for pine snakes. For example, mercury and selenium were three times as high in snakes from Poplar Creek (Oak Ridge, a contaminated site) than in pine snakes in the NJ Pine Barrens (Campbell et al. 2005). Recently, Schneider et al. (2013) reported the concentrations of metals from 11 species of snakes (including 5 of our studies), and concentrations from these studies were generally similar to those reported for pine snakes in this paper.

The only other study of metal concentrations in snakes from New Jersey examined concentrations in Northern Water Snakes (*Nerodia sipedon)* living in the Raritan River that flows into the Atlantic Ocean. Although pine snakes live on land and Water Snakes live in the water, both obtain their contaminants primarily from their prey (the Raritan River water is not contaminated as it is the local source of drinking water and contaminant levels are assessed regularly). The concentrations in the skin of water snakes, for example, were very similar to those in pine snakes from 2010 to 2016, except for mercury and selenium (Burger et al. 2007). Mercury concentrations in skin of water snakes averaged 159 ± 23 compared with 41 ± 7 for pine snakes. Selenium concentrations in skin of water snakes averaged 725 ± 71 compared with 182 ± 36 for pine snakes. These higher levels may reflect external contamination (Burger 2002).

Concentrations that Cause Effects

Mercury is the metal contaminant of most concern in vertebrates because mercury bioaccumulates as vertebrates age and is usually higher in large, top trophic level

Table 4 Correlation between tissues for metal levels in pine snakes from New Jersey

	Skin and				Muscle and			Liver and		Heart and Kidney
	Muscle	Liver	Kidney	Heart	Liver	Kidney	Heart	Kidney	Heart	
Arsenic	NS	NS	NS	NS	−0.32 (0.08)	NS	NS	NS	0.59 (0.005)	NS
Cadmium	0.41 (0.02)	0.41 (0.03)	0.48 (0.01)	NS	0.32 (0.08)	NS	NS	0.39 (0.04)	NS	NS
Chromium	0.34 (0.04)	0.39 (0.03)	NS	NS	0.39 (0.03)	NS	NS	0.45 (0.02)	NS	NS
Lead	0.41 (0.01)	NS	NS	0.35 (0.07)	0.36 (0.05)	0.46 (0.02)	0.45 (0.02)	0.59 (0.003)	0.38 (0.06)	0.52 (0.01)
Mercury	0.56 (0.0006)	0.37 (0.04)	0.50 (0.009)	0.58 (0.003)	0.50 (0.005)	0.58 (0.003)	0.66 (0.0006)	0.50 (0.009)	NS	0.41 (0.05)
Selenium	NS	0.64 (0.0004)	0.35 (0.07)	NS	NS	NS	NS	NS	NS	0.38 (0.07)

Given are Kendall tau correlations (*p* values)

Table 5 Mean moisture content of pine snake samples, and conversion factor for converting metal levels from wet weight to dry weight (wet weight × CF = dry weight)

Tissue	%Moisture	Conversion factor (CF)
Skin	74.1	3.85
Muscle	73.1	3.72
Liver	74.6	3.94
Kidney	76.8	4.31
Heart	73.8	3.82

Samples were dried at 40° for 48 h and then reweight

predators (reviewed in Schneider et al. 2013; Burger and Gochfeld 2016). Metal concentrations in snake tissues are usually obtained through the food chain from their prey (Campbell and Campbell 2001; Burger et al. 2006; Schneider et al. 2013). In some cases, snakes exhibit higher metabolic rates (Hopkins et al. 1999), which might make them more vulnerable to a rapid exposure to contaminants in prey. Reviews by Campbell and Campbell (2001) and Schneider et al. (2013) indicated that there were no laboratory studies with snakes that determined the concentrations at which adverse effects occur. Laboratory studies examining adverse effects are not usually completed, because the U.S. Environmental Protection Agency does not require testing on snakes (Campbell and Campbell 2001).

Use of Pine Snakes as Indicators of Metals in the Environment

Snakes can be useful indicators of environmental contamination, because many are large predators that can live up to 25–30 years (Burger and Zappalorti 2011; W. Brown, personal communication), providing an opportunity for bioaccumulation (Campbell and Campbell 2001). Concentrations in the tissues of sakes reflect the concentrations in the prey items that they consume, and when a larger predator eats them, the snakes reflect the concentrations obtained by the predator. Because large snakes are generally carnivorous, examining concentrations of metals in snakes can provide information about contaminant concentrations in the prey that they consume (small mammals) for potential risks to consumers who eat them (mammals, other snakes, hawks) and the potential risks to pine snakes themselves. The variation in metal concentrations both within a species and among different species living in the same geographical area can provide information on bioaccumulation patterns and exposure.

Some snakes, such as water snakes or garter snakes, are sufficiently common that they can be collected specifically for contaminant analysis without impacting the stability of populations (Burger et al. 2006). Both are aquatic generalists. Other species, however, such as pine snake and the giant garter snake, are threatened or endangered and cannot justifiably be collected for metal analysis. Scientists rely on finding dead snakes to examine metal concentrations (Wylie et al. 2009). Snakes found dead may not be a random sample of the population if metal toxicity affects behavior, snakes exposed to contaminants may be less able to avoid predators, or they spend more time on roads.

In the present study, the concentrations of mercury in skin were significantly correlated with the concentrations in internal tissues for all metals. Because mercury is the main contaminant of concern for wildlife and for reptiles (Schneider et al. 2013), it is important that skin can be used as an indicator, because it reflects concentrations in other tissues. Because snakes, particularly large snakes, such as pine snakes, are top-level predators, they eat prey that also accumulate metal concentrations. In the Pine Barrens, pine snakes are one of the primary predators. This suggests that pine snakes (shed and skin) can be used as an indicator of internal concentrations.

Further, these results provide a nondestructive method for scientists and managers to track regularly the concentrations of metals in snakes in the Pine Barrens. Strategies for assessing contaminants in snakes can be developed that rely on the public to collect snakes found dead along highways as a way of both tracking mortality and tracking metal levels. Programs that (1) allow people to collect snakes on highways, recording dates, times, and conditions, (2) encourage freezing of specimens for later analysis, (3) and reward cooperators with periodic information on the program might allow state agencies to accumulate information on snake mortality and morphometric, as well as samples for biomonitoring of heavy metals.

Methodological Considerations

Some comments on methodological issues with contaminant concentrations in biota are warranted. One of the major difficulties with contaminant studies is the lack of uniformity in the tissue samples collected, as well as the metals examined, making comparisons among species and geographical regions difficult. While some tissues can be collected noninvasively (e.g., feathers and hair), others require invasive collection (e.g., blood) or even lethal collection (e.g., liver). However, examining different tissues is the only method to determine what is happening internally and to provide an indication of potential harm to the snakes. Such studies are essential before any noninvasive tissues can be used routinely and interpreted with confidence. With large, rare snakes that may be threatened or endangered, killing healthy individuals is unwise (in terms of possible effects on populations), but collection of recently killed snakes from paved roads provides an alternative. Most studies that examine concentrations of metals are conducted with snakes from known contaminated sites to determine if there is bioaccumulation, and then investigators usually collect only blood and tail clips (Drewett et al. 2013). Furthermore, metal concentrations in marked snakes also can be examined by using either blood or tail clips (Burger et al. 2006) and could be examined over many years.

One of the commonest methods currently used to assess contamination is to either examine blood or to examine muscle from tail clips. Blood represents acute exposure, whereas tail clips represent cumulative exposure. One of the difficulties with tail clips is the consistency of sampling, because the amount of tail material (e.g., the length of the clip) will determine the quality of the tissue and the relative amount of skin/muscle/bone, and perhaps the amount of infused blood remaining may vary. Because skin tissue has higher concentrations of metals than muscle (Burger 1992), this may be an important consideration. We were unable to examine the relationship between blood concentrations and internal tissues because the blood samples were taken from different snakes. Snakes found freshly dead do not have usable blood, and all blood samples in this study were taken from live snakes at the end of hibernation.

In conclusion, the data from this study indicate road-killed pine snakes can be used to assess the metal exposure of pine snakes and that these data can be used to assess both temporal and spatial patterns of metal exposure without needlessly killing snakes. Shed skins are also useful for bioindication.

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