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USING RACCOONS AS AN INDICATOR SPECIES FOR METAL ACCUMULATION ACROSS TROPHIC LEVELS: A STABLE ISOTOPE APPROACH

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Abstract: The fact that raccoons (*Procyon lotor*) are an opportunistic omnivore has severely complicated interpretations of contaminant uptake patterns due to the inability to determine the trophic position an individual occupies. Moreover, few studies have examined the relationships between heavy metal bioaccumulation and trophic structure, especially in the terrestrial environment. In this study, the stable isotopes of nitrogen were used to characterize the feeding habits of the raccoon at the population level and to determine whether metal burden was related to trophic feeding structure within a well-defined ecosystem. Raccoon populations were isotopically distinct, and significant positive relationships existed between some trace element contents and $\delta^{15}N$ of muscle when site was used as a covariable in a statistical model. Although the transfer of metals through terrestrial ecosystems is complex, our study showed that some of the variation in contaminant body burdens in raccoon populations can be attributed to trophic feeding position and that $^{15}N/^{14}N$ ratios of muscle tissue provide a quantitative measure of this process. The potential for using omnivores such as the raccoon, as a sentinel species for contaminant studies, should be explored further since the ambiguity of the relative trophic level an animal occupies can be directly estimated. This provides a more extensive sampling across trophic levels using a single species, which can have broad consequences for ecological risk assessments.

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The stable isotope composition of biological materials provides insights into the life histories of wildlife species (Hobson and Wassenaar 1997, Gannes et al. 1998, Kelly 2000). It has been demonstrated that animal tissues are enriched in ^{15}N in relation to their diets (DeNiro and Epstein 1981). The differences in isotopic composition between any tissue compartment of an animal and diet is represented by a tissue-diet enrichment factor $\varepsilon_{\text{tissue-diet}}$, where $\varepsilon_{\text{tissue-diet}} \approx \delta_{\text{tissue}} - \delta_{\text{diet}}$ and "δ" is the delta value for the isotope of interest (Craig 1953). The partitioning of nitrogen isotopes can be viewed as a steady state mass balance in most organisms in which excretory products are depleted in ^{15}N and tissues are enriched in ^{15}N compared with the diet. This enrichment is expressed for every trophic step in the food web of an ecosystem, and results in higher-level consumers having nitrogen isotope ratios that are approximately 3‰ greater in ^{15}N compared with their prey (Minagawa and Wada 1984, Romanek et al. 2000).

Many studies have focused on measuring $^{15}N/^{14}N$ ratios in blood, liver, muscle, and/or bone collagen. Of these, blood has a relatively short turnover time (hours to days), skeletal collagen has a turnover time that approaches a year, while muscle records information on a time scale of up to a month depending on the foods assimilated and the metabolic activity of the host (Tieszen et al. 1983, Hobson and Sealy 1991, Hobson and Clark 1992). For this study, we focused on measuring the $\delta^{15}N$ of raccoon muscle tissue to determine the trophic range over which this species was feeding in 5 areas of a well-defined ecosystem that was differentially exposed to environmental contaminants.

Several characteristics of raccoons make them an ideal sentinel species for contaminant studies:

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(1) high population levels with an extended range throughout North America in a variety of habitats; (2) a broadly omnivorous diet that includes components of both terrestrial and aquatic food chains; (3) a propensity to utilize human-altered habitats in combination with an ability to move freely in and out of waste sites; and (4) a proclivity to travel extended distances, which makes them potential agents of contaminant movement and dispersal. However, the fact that raccoons are an opportunistic omnivore severely complicates interpretations of contaminant uptake patterns because of the inability to accurately quantify the integrated trophic position an individual occupies.

In this study, we explore the use of stable isotopic analysis as a tool to quantify the feeding habits of this omnivorous species at the population level and determine the role of trophic status in controlling some of the variability associated with contaminant burdens. Specifically, our objectives were to (1) quantify the range of $\delta^{15}N$ in raccoon muscle from 5 different sites on and near the U.S. Department of Energy's Savannah River Site (SRS); (2) determine whether the trophic position of raccoons from different sites could be identified using the well-known increase of approximately 3‰ in $\delta^{15}N$ per trophic step; (3) determine whether contaminant burdens can be better understood among sites when trophic position is estimated based on 3 possible models:

(a) Metal accumulation is a function of $\delta^{15}N$, site, and a higher order interaction term:

$metal \cong f(\delta^{15}N, site, \delta^{15}N \times site)$;

(b) Metal accumulation is a function of the $\delta^{15}N$ and site, where $\delta^{15}N$ does accumulate in the same way at every site:

$metal \cong f(\delta^{15}N, site)$;

(c) Metal accumulation is a function of $\delta^{15}N$ and does not change based on site:

$metal \cong f(\delta^{15}N)$.

STUDY AREA

The SRS is a 778-km^2 former nuclear production and current research facility located in west-central South Carolina, USA (33.1°N, 81.3°W; Fig. 1) that was closed to public access in 1952. In 1972, the entire SRS was designated as the nation's first National Environmental Research Park to provide tracts of land where the effects of human impacts upon the environment could be studied (Davis and Janecek 1997). Much of the suitable forested area of the SRS is managed primarily for commercial timber production (pine).

Fig. 1. Map of the U.S. Department of Energy Savannah River Site, South Carolina, USA, showing wetlands and river drainage systems. Numbered circles indicate raccoon sampling locations.

More than 20% of the SRS is covered by wetlands, including bottomland hardwoods, cypress–tupelo swamp forests, creeks, streams, ponds, Carolina bays, and 2 large cooling reservoirs (Par Pond–Pond B and L-Lake).

We collected raccoons from 4 locations on the SRS (Fig. 1)—from an 87-ha former reactor cooling reservoir (Pond B) and a disturbed stream flood plain (Steel Creek) directly contaminated by ^{137}Cs releases. Both of these systems have been intensively studied regarding the bioaccumulation of ^{137}Cs in resident flora and fauna (Brisbin et al. 1974, 1989; Evans et al. 1983; Gladden et al. 1985; Whicker et al. 1990; Gaines et al. 2000). Pond B (part of the Par Pond reservoir system) received cooling water discharges from the SRS R-Reactor (shut down in 1964) that were contaminated with ^{137}Cs from leaking reactor fuel elements. This reservoir originally received water, and current water levels are maintained from the Savannah River, which has been shown to have elevated levels of mercury (Sugg et al. 1995). Although the original sources of the mercury in the river are not high, water pumped into Pond B to counter loss by evaporation and seepage has led to increased concentrations in the reservoirs over several decades.

The Steel Creek watershed drains into an inundated riverine swamp delta that is contiguous to

the Savannah River. Two production reactors discharged effluents into Steel Creek containing cooling water mixed with purge water from basins used to store irradiated reactor fuel and target assemblies. ^{137}Cs that leaked from defective experimental fuel assemblies was discharged into Steel Creek via this purge water (Ashley and Zeigler 1980). Further, Steel Creek is the drainage source for the L-Lake reactor-cooling reservoir that was constructed in 1985. This reservoir also has elevated levels of mercury for the same reasons as Pond B.

The third site on the SRS that was selected for this study also was directly affected by plant operations, via a coal-fired power plant (D-Area basins) that discharges sluiced fly and bottom ash into a series of open settling basins that subsequently drains into a 2-ha swamp and into Beaver Dam Creek, a tributary of the Savannah River. Past investigations of the D-Area basins and nearby Beaver Dam Creek have found enrichment of Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Se, and Zn in water, sediments, and biota (Cherry and Gutherie 1977, Evans and Giesy 1978, Alberts et al. 1985, Sandhu et al. 1993, McCloskey and Newman 1995, Rowe et al. 1996).

The fourth site on the SRS, an undisturbed natural stream flood plain system (Upper Three Runs–Tinker Creek area), was not directly contaminated by plant operations. Upper Three Runs Creek (UTR) has been used previously as a standard reference site for comparing other upper coastal plain stream areas of the southeastern United States. Raccoons also were collected from nearby public hunting grounds near the SRS (Fig. 1). These sites included an area located approximately 8 km west-northwest of SRS (Jackson), an area located approximately 15 km northwest of SRS (Beech Island), and an area approximately 3 km from the southern SRS boundary (Creek Plantation). All 3 hunting areas are part of the bottomland hardwood floodplain ecosystem of the Savannah River, which extends into the SRS along the site's southwestern border, and are considered a single site.

METHODS

Stable Isotope Analysis

Elemental analysis–isotope ratio mass spectrometry was employed to measure the total nitrogen content and the $^{15}N/^{14}N$ ratio from individual raccoon muscle samples (Barrie and Prosser 1996). All measurements were performed in the Stable Isotope Laboratory at the Institute of Ecology, University of Georgia, Athens, USA. Prior to isotopic analysis, we homogenized muscle tissue by grinding freeze-dried samples with a mortar and pestle. Approximately 2 mg samples of ground muscle were loaded into individual pre-cleaned tin capsules and weighed to ± 1 μg using an ultramicrobalance. We then placed capsules in the autosampler of a Carlo Erba Elemental Analyzer (NA1500) attached to a continuous flow isotope ratio mass spectrometer (Finnigan Delta+). Samples were combusted to N_2 in oxidation–reduction furnaces, purified by gas chromatography, and measured for $^{15}N/^{14}N$ content on the mass spectrometer. An internal $N_2(g)$ working standard was admitted prior to each sample combustion for calibration to the international AIR standard (Mariotti 1983). Stable isotope ratios are reported in per mil units (‰) using standard delta (δ) notation (Craig 1957). External working standards of bovine liver and acetanilide were analyzed to determine external precision; collectively, they were reproducible to better than ± 0.15‰ (1 σ).

Metal Analysis

At the Environmental and Occupational Health Sciences Institute, tissues were digested in Ultrex ultrapure nitric acid in a microwave (MD 2000 CEM), using a digestion protocol of 3 stages of 10 min each under 50, 100, and 150 pounds per square inch (3.5, 7, 10.6 kg/cm^2, respectively) at 70× power. Digested samples were subsequently diluted in 100 ml deionized water. All laboratory equipment and containers were washed in 10% HNO_3 solution prior to each use.

We analyzed metals (As, Cd, Cr, Cu, Mn, Pb, Se, Sr) by inductively coupled plasma mass spectroscopy (ICP-MS). Chromium was not speciated to Cr(III) and Cr(VI). Mercury was analyzed by cold vapor atomic absorption spectroscopy (HGS-4). We used cold vapor for mercury because it proved difficult to analyze on the ICP-MS. The ICP-MS was calibrated using Custom Grade standards at the beginning of each batch, and after every fifteenth sample. Detection limits were As = 0.2, Cd = 0.02, Cr = 0.08, Cu = 0.2, Hg = 0.2, Mn = 0.09, Pb = 0.015, Se = 0.7, and ^{88}Sr = 0.02, expressed in parts per billion (ng/g) on a wet weight basis. An EPA standard was run at the beginning of each run for initial calibration verification. All specimens were run in batches that included blanks, a suite of calibration standards and spiked sample specimens. The accepted

recoveries for spikes ranged from 80% to 120%; no batches were outside of these limits. The coefficient of variation (CV) on replicate samples ranged from 2% to 8%.

^{137}Cs Determinations

We determined ^{137}Cs count rates of wet muscle tissues using a Packard Auto-Gamma A5530 counting system (Packard Instrument, Meriden, Connecticut, USA) with a 7.62-cm thallium-activated NaI crystal of through-hole design with a counting window of 550–760 keV. Accuracy of the instrument was assessed by calibration prior to every counting sequence using a certified calibration standard. Counting time per sample was 60 min. Counting times were based on preliminary analyses of the sample count rate for all samples and sample masses, relative to resultant minimal detectable concentrations (MDC). To estimate tissue wet mass ^{137}Cs concentrations (Bq/g), sample gross count rates were adjusted for background count rates. We then compared the adjusted count rates of samples to similarly adjusted count rates of aqueous standards approximating the sample geometry and containing known quantities of ^{137}Cs. We recorded background count rates following every third sample. This also provided a measure of the precision of the instrument over the analysis period. Count rates of standards were determined daily. Standard count times were of identical duration to count times of samples to which they would be compared. The MDC were calculated following procedures described by Currie (1968).

Raccoon Trapping and Sampling

We collected raccoons (n = 43) on the SRS between December 1996 and June 1998 using baited traps (manufactured by T. Fox, Batesburg, South Carolina, USA) set in the afternoon and checked the following morning. Raccoons were transported to the laboratory, euthanized, and dissected immediately. We used only male raccoons for this study to control for possible variation due to sex and to prevent the removal of pregnant females or females with young from the population. An additional 25 raccoons were collected offsite but near the SRS during the statewide hunting season between January and February 1997 and frozen individually in labeled plastic bags until dissection. Gastrocnemius muscle tissue (10–20 g) was removed from each individual and frozen for later analyses. Individuals could not be definitively aged, but no yearlings were used in the analyses. Tooth-wear examination indicated that adults probably were of the same age structure, and other measurements such as weight were taken for an overall body condition index.

Statistical Analyses

We first examined metal and isotope distributions using Shapiro-Wilk statistics (PROC UNIVARIATE, version 6.12; SAS Institute 1998). Tests of hypotheses that these data were random samples from normal distributions and tests of homogeneity of variances were rejected ($P < 0.05$), and stem-and-leaf plots suggested a log-transformation of data prior to analysis. Any analysis using ^{137}Cs included only raccoons from locations contaminated from site operations (Pond B, Steel Creek). Analyses using Hg were run with and without samples from the Ash Basins site for reasons discussed below.

We examined differences among sites for δ^{15}N via analysis of variance (ANOVA) models (PROC GLM; SAS Institute 1998) with appropriate Bonferroni corrections. To examine the relationship between the metal burdens in raccoon muscle tissue to its δ^{15}N value with the simultaneous effect of location, we used analysis of covariance models (ANCOVA; PROC GLM; SAS Institute 1998) and heterogeneity of slopes models. If the interaction between location and δ^{15}N was not significant, we dropped the interaction term from the model and used a standard ANCOVA. If location was not significant, we used a linear regression to determine the relationship between metal tissue concentration and δ^{15}N. For all tested models, Type III (partial) sums of squares and associated F-statistics were interpreted and least-squares means procedures were used to provide estimates of dependant variables that were adjusted for all effects in the models and to provide mean separation tests. All statistical tests were considered significant at $P \leq 0.05$. Means and standard errors are presented as back-transformed values of log least-squares means estimates (i.e., geometric means).

RESULTS

The range of δ^{15}N values for raccoon muscle tissue was relatively large (4.28 to 13.68‰), indicating that raccoons probably fed over a range of trophic levels (approx. 3). Least-squares means of raccoons from different locations on and near the SRS ranged from 6.72 to 10.03‰, representing approximately 1 trophic level across sites.

Table 1. Comparisons of least-squares mean (LSM) muscle $\delta^{15}N$ values (‰) of raccoons between locations on and near the Savannah River Site, South Carolina, USA. Values for mean and upper and lower 95th percentiles were calculated on log-transformed data and back transformed. Bonferroni adjustments were performed for multiple comparison tests. Differences among sites were significant by analysis of variance ($F_{4, 63} = 7.03$, $P = 0.0001$).

						$p > \|T\|$ H_0:LSM(i) = LSM (j)			
			95th percentiles			j			
Location	Raccoons (n)	LSM $\delta^{15}N$	Upper	Lower	i	1	2	3	4
Ash Basins	12	10.03	11.36	8.85	1				
Offsite	25	8.06	8.80	7.40	2	0.067			
Pond B	9	6.96	8.04	6.02	3	0.004	0.900		
Steel Creek	10	9.33	10.69	8.13	4	1.000	0.850	0.054	
Upper 3 Runs	12	6.72	7.61	5.92	5	0.000	0.212	1.000	0.010

The range of $\delta^{15}N$ was 6.72 to 13.68‰ for the Ash Basins; 5.60 to 12.13‰ for Offsite; 5.87 to 11.75‰ for Pond B; 6.60 to 12.21‰ for Steel Creek; and 4.28 to 8.84‰ for Upper Three Runs. Although these ranges overlap, $\delta^{15}N$ differed by location (ANOVA: $F_{4, 63} = 7.03$, $P = 0.0001$; Table 1; Fig. 1). Summary statistics for metal concentrations are reported in the Appendix.

The effect of $\delta^{15}N$ with location on As concentration in muscle tissue was significant with a significant interaction effect. There was a significant effect of $\delta^{15}N$ and location on concentrations of Sr, Mn, Cr, and Se (individual ANCOVA models) in raccoon muscle tissue, with no interaction effect on the model. No relationship occurred between Hg concentrations in raccoon muscle tissue with $\delta^{15}N$, but there was a location effect and a significant interaction effect. However, when data from the Ash Basins were removed from the model, there was a significant effect of $\delta^{15}N$ and location on concentrations of Hg, with no interaction effect. The effect of $\delta^{15}N$ on Cd and Cu was marginally significant ($P = 0.06$ and $P = 0.09$, respectively) when location had a significant effect on the model. There was no relationship between Ni concentrations with location and/or $\delta^{15}N$. Pb concentrations were dependent on $\delta^{15}N$; however, location did not significantly contribute to the model. Finally, no relationship occurred between ^{137}Cs concentrations with location and $\delta^{15}N$, but there was a marginal relationship between ^{137}Cs concentrations and $\delta^{15}N$ ($P = 0.06$). For all significant models, the contaminant in question tended to increase as $\delta^{15}N$ (presumably trophic position) increased (Table 2).

DISCUSSION

The stable isotope composition of animal tissues provides an understanding of the life histories of wildlife species, through the enrichment in ^{15}N in relation to their diet, that was previously ambiguous. Clearly, individual raccoon populations on and near the SRS are isotopically distinct. These differences could be due to intrinsic differences in the $\delta^{15}N$ of the nitrogen base among site populations, or it may be due to trophic level effects, where different individuals occupy different trophic positions in the food web of a coherent ecosystem. However, since these sites are spatially close and geomorphologically similar, the former explanation is unlikely. This conclusion is supported by collections of food resources at the SRS. Previous studies have shown only relatively small differences in $\delta^{15}N$ among similar food items across the site (S. F. Pearson, Washington Department of Natural Re-

Table 2. General Linear Model results (*F*-values shown with *P*-values in parentheses) for the effect of raccoon muscle $\delta^{15}N$ values and location on raccoon muscle metal concentrations, Savannah River Site, South Carolina, USA. Interaction effects are shown if significant, otherwise standard ANCOVA models were used. Model results are reported using Type III sums of squares. If the effect of site was not significant, it was dropped from the model and a standard linear regression was used. For all significant models, the contaminant in question tended to increase as $\delta^{15}N$ muscle tissue levels increased.

Metal	Site	$\delta^{15}N$	Site × $\delta^{15}N$
Se	18.61 (<0.001)	22.83 (<0.001)	n/a
Hg[a]	2.81 (0.034)	0.71 (0.401)	2.56 (0.048)
Hg[b]	13.66 (<0.001)	4.89 (0.032)	n/a
As	5.25 (0.001)	15.31 (<0.001)	3.62 (0.011)
Mn	7.49 (<0.001)	10.77 (0.002)	n/a
Ni	1.98 (0.108)	2.16 (0.147)	n/a
Cu	4.82 (0.002)	3.00 (0.088)	n/a
Sr	4.00 (0.006)	5.83 (0.019)	n/a
Cr	15.09 (<0.001)	4.32 (0.042)	n/a
Cd	22.75 (<0.001)	3.56 (0.064)	n/a
Pb	n/a	8.32 (0.005)	n/a
Cs-137	n/a	4.08 (0.061)	n/a

[a] Model run using all sites.
[b] Model run without the Ash Basins site.

Table 3. $\delta^{15}N$ levels (‰) of various potential raccoon food items (Kinard 1964, Johnson 1970) collected during the trapping period from the Savannah River Site, South Carolina, USA.

Species	*n*	$\delta^{15}N \pm SD$
Mosquito fish (*Gambusia holbrooki*)	3	8.6 ± 0.30
Bluegill (*Lepomis macrochirus*)	3	10.8 ± 0.11
Largemouth bass (*Micropterus salmoides*)	5	12.4 ± 0.47
Brown bullhead (*Ameiurus nebulosus*)	1	10.01
Snails (no shell; Planorbidae)	3	5.0 ± 0.20
Eastern elliptio mussel (no shell; *Elliptio complanata*)[a]	5	7.47 ± 0.32
Adult beetles (Coleoptera)	3	4.9 ± 0.71
Dragonfly larvae (Odonata)		
Species 1	3	4.3 ± 0.20
Species 2	3	3.8 ± 0.02
Crayfish (Cambaridae)	4	4.43 ± 3.16
Persimmon (*Diospyros virginiana*)[b]	2	3.55 ± 0.49

[a] JV. MacArthur, Savannah River Ecology Laboratory, unpublished data.
[b] S. F. Pearson, Washington Department of Natural Resources, unpublished data.

sources, unpublished data). Further, the inherent omnivorous character of the species, the compilation of ^{15}N from SRS data sources of potential food items based on a study of the foraging habits of raccoons on the SRS (Kinard 1964), and the distribution of indigenous soil and vegetation among sites, make the latter hypothesis more likely (Table 3).

In this study, many potential factors undoubtedly added variance to the relationships observed among the data. All these factors tend to obscure the significance of relationships between $\delta^{15}N$ and metal concentrations in muscle tissue. For instance, if it were shown that age (e.g., differences in 2–3 yr olds vs. 3–4 yr olds) could be used to explain metal accumulation in muscle tissue, it should not confound any of the conclusions of this study because it would have to be highly correlated to ^{15}N to compromise this relationship. That is, even if higher metal loads were observed in older individuals, $\delta^{15}N$ also would have to be higher in those individuals, and it was clear that $\delta^{15}N$ patterns were associated with site.

The possibility exists that seasonal differences in water availability could be correlated to $\delta^{15}N$, and therefore explain the relationship between $\delta^{15}N$ and metal content. This is highly unlikely because rainfall is variable and tends not to be seasonal in this area of the southeastern United States. Kelly (2000) classified habitats as either xeric or mesic to demonstrate the correlation between $\delta^{15}N$ and water availability. While this relationship was shown to be robust at a global scale, it required the comparison of organisms from environments of very different water availability and habitat. Further, muscle was chosen in our study for its high turnover rate (approx. 1 mo, which mimics the trapping regime for each site) and, therefore, the patterns that are being expressed in the models are most likely to be from food-chain effects.

During our study, raccoons were randomly sampled within sites to control for confounding effects. Further, we tested for the influence of the most likely confounding variables—date or season and body weight (as a function of condition)—on the $\delta^{15}N$ of muscle tissue. Both variables proved to be highly insignificant ($P > 0.30$). In this study, no yearlings were used in the analyses, and tooth-wear examination indicated that adults were probably of the same age structure (2–4 yr old). Further investigation of age structure is extremely difficult in mild southeastern climates due to inconsistent ring annuli formation in tooth cementum (Grau et al. 1970). Additionally, raccoon home ranges on the SRS in an on-going telemetry study (C. S. Boring and K. F. Gaines, unpublished data) have shown that male home ranges were an average of 216 ha ($n = 10$), which would preclude them from overlapping in resource utilization between study locations. Based on these observations, the nitrogen isotope ratios most likely represent the relative trophic position at which individuals within the raccoon populations were feeding. This is supported by observed $\delta^{15}N$ values, which indicate that Pond B and Upper Three Runs raccoons consistently consumed foods at lower trophic positions than raccoons from the Ash Basins and Steel Creek. Since raccoons are opportunistic omnivores, these findings suggest that the $\delta^{15}N$ of muscle reflects assimilated food sources that vary by location on and near the SRS.

Metal levels in muscle, liver, heart, spleen, and kidney from these raccoons were shown to differ between locations (Burger et al. 2002). Moreover, the metal levels in muscle tissue had large ranges (Appendix). Since the fugacity (partitioning of the compounds in the environment; Mackay and Paterson 1981) determines the availability of the substance in the habitat occupied by the organism, it is extremely important to characterize the variation of the contaminants at these sites. Stable isotope patterns helped explain how these metals contributed to the metal burdens of raccoons from different locations. Several studies

have demonstrated the utility of stable nitrogen isotope analysis in estimating uptake of environmental contaminants, including organochlorides, polychlorinated biphenyls, and dioxins (Broman et al. 1992, Rolff et al. 1993, Kidd et al. 1995, Muir et al. 1995, Jarman et al. 1996). Yet few studies have examined the relationships between nitrogen stable isotope ratios and heavy metal contaminants, especially in the terrestrial environment.

Bioaccumulation is defined as the net consequence of uptake, transformation, and elimination of a toxicant in an individual; and toxicant transfer from 1 individual to another in the course of trophic interactions is part of bioaccumulation. Biomagnification is said to occur if the concentration of toxicant increases during successive trophic transfers (Newman 1994). In this study, we define intraspecific biomagnification to be a direct relationship between toxicant burden and trophic position of individuals within a population of a single species. For the most part, studies focusing on metal accumulation in wildlife have shown some level of bioaccumulation in muscle tissue. However, most of the time, no consistent pattern is found in the distribution of contaminants in ecosystem food webs. More specifically, it is unclear whether metal concentrations increase as trophic position increases in a terrestrial environment (i.e., biomagnification). This may be due in part to the differences in foraging strategies of the consumers in these terrestrial environments and the inability to accurately recognize the trophic position of organisms in an ecosystem. Understanding the movement of contaminants through trophic levels within a single organism can control for some of this variation.

Metal Bioaccumulation

Previous investigations of the Ash Basins site have documented elevated levels of Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Se, and Zn in water, sediments, and biota (Cherry and Gutherie 1977, Evans and Giesy 1978, Alberts et al. 1985, Sandhu et al. 1993, McCloskey and Newman 1995, Rowe et al. 1996). When these metals and metalloids are present at such high levels, they may very well interact with one another and influence their bioavailability in this ecosystem. For example, in this study, no relationship occurred between Hg accumulation and $\delta^{15}N$ of muscle tissue when the entire data set was used. However, the significant interaction effect of site and $\delta^{15}N$ with a non-significant effect of $\delta^{15}N$ on the model led us to explore the data further. When examining the slopes of each site, the Ash Basins site was not significant, and its overall slope moved in a different (inverse) direction than the other significant slopes. Based on this examination, we dropped the Ash Basins site from the model. When this site was removed, a clear relationship existed between Hg and $\delta^{15}N$. We hypothesize that the unique environment of the Ash Basins with multiple elevated trace elements may complicate Hg uptake in vertebrate biota.

The synergistic effect of multiple contaminants in the Ash Basins ecosystem may act to change the toxic effects of exposure to single pollutants. For example, the toxicity of Hg and Se are reduced when organisms are exposed or dosed with these 2 metalloids simultaneously (Shamberger 1981, Pelletier 1985, Cuvin-Aralar and Furness 1991). Although no relationship was found between Se and Hg in our study, this interaction possibly could affect Hg uptake. Another possibility that could affect the bioavailability of Hg in the Ash Basins is dissolved sulfate concentration. The dissolved sulfate concentration of Ash Basins water is relatively high (approx. 50 ± 10 ppm; Alberts et al. 1985, Westinghouse Savannah River Company [WSRC] 2000), and up to 10,000 ppm for seeps and ponded waters in the area (Carlson 1990, WSRC 2000), while sulfate in other waters of the SRS such as Par Pond, Steel Creek, and Upper Three Runs Creek averages >5 ppm (Alberts et al. 1985, Snodgrass et al. 2000). It has been shown that Hg contamination in estuarine and saltwater environments can be mitigated by sulfate-reducing bacteria that produce sulfide (Windom et al. 1976), which strongly binds with Hg in aquatic environments (Stumm and Morgan 1996). However, some studies suggest that elevated dissolved sulfate can stimulate the methylation of Hg by sulfate-reducing bacteria (Winfrey and Rudd 1990, Gilmour et al. 1992), which might increase Hg bioavailability. The relatively high sulfate concentration in Ash Basins waters may have reduced the bioavailability of Hg for transfer through the food web.

Another example of how metalloid bioavailability in the Ash Basins differed from other sites was expressed in the significant relationship between As concentration and $\delta^{15}N$ in muscle tissue. There was a significant interaction effect in the general linear model driven by the wide range of As in the Ash Basins, which resulted in a steeper slope than any of the other sites. It has

been documented that levels of As in banded water snakes (*Nerodia fasciata*) from this site are the highest ever documented in a reptile or amphibian (Hopkins et al. 1999), indicating that As is highly bioavailable in this system and preferentially accumulates through the food web of this ecosystem.

Heavy-metal accumulation across trophic levels is not well understood, especially in terrestrial ecosystems. In most cases, the contaminant must reside in a mobile form for transfer from the soil to plant and from plant to animal. This passage of metals from the soil into plants represents the gateway for metal entry into the food web. Plant processes can influence the chemical behavior of contaminants from root absorption through transport into woody and leaf tissues, and into the seeds and fruits (Hunter et al. 1987*a,b,c*). Thus, exposure to contaminants may be strongly linked to the kind of plant material an organism ingests (e.g., leaf vs. fruit). Contaminant studies have relied on comparisons among a variety of target species, which confound interpretations due to dietary variations and differences in interspecific physiologies. Further, it is unclear how contaminant exposure may affect the foraging strategy of the study organism and its animal prey base. A better understanding of metal biochemistry and behavior in physiological systems will greatly improve interpretations regarding the presence of contaminants in biological tissues and the transfer of these contaminants across trophic gradients of impacted ecosystems.

MANAGEMENT IMPLICATIONS

Accumulation of toxic metals within an organism in terrestrial ecosystems is a complex process related to both abiotic and biotic factors. For most wildlife species, as in this study, exposure to multiple contaminants usually is the norm, and the potential interaction effects of contaminants can alter the form, storage, metabolism, and excretion of a compound (Peterle 1991). Biomagnification within a species (intraspecific biomagnification) may be very different than transfer between species due to physiological responses of the ingested contaminants. Examining intraspecific biomagnification of contaminants has been almost impossible prior to the advent of the stable isotope methodologies. Such relationships could not be documented without extended feeding trials of captive animals known to occur in a given food web, which confound studies that utilize wildlife species for ecosystem management. Our study shows evidence that the trace element burdens of raccoons were related to trophic position within an impacted ecosystem and that stable isotopes can be used to better understand the distribution of contaminants in and among populations. The potential for using omnivorous game such as the raccoon, as a sentinel species for contaminant studies, should be explored further since the ambiguity of the relative trophic level an animal occupies can be directly estimated. In addition, the omnivorous nature of the raccoon provides a broader sampling of a single species across trophic levels. Thus, nonlinearities of trophic transfer within and between contaminants are more easily determined. This can have broad consequences for both human-based and ecological risk assessments.

As urbanization continues, hunters are becoming more concerned about what contaminants may be contained in the game they eat. In the Southeast, raccoons are hunted for both meat and fur (Gaines et al. 2000). In South Carolina, the raccoon-hunting season usually is from mid-September to mid-March, with no bag or possession limit. Thus, a diligent hunter who ate the meat could legally consume as much raccoon meat as desired. Since the raccoon is easy to sample (either from hunters, trappers, or road kill), using it as a sentinel species is advantageous to risk assessors and the hunting public. Stable isotope analyses can provide a cost-effective measure of determining whether a consumption risk exists with some limited knowledge of the trace element levels in an area. Further, methods as outlined in this study give researchers the opportunity to use omnivores as indicator species of environmental health. Future management efforts may benefit from the trace element and $\delta^{15}N$ analyses of selected ecosystem components, and inorganic–organic metal uptake processes to further examine the role of metal biomagnification in ecotoxicological investigations.

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Appendix on next page.

Appendix. Summary statistics for metal concentrations in raccoon muscle tissue, Savannah River Site, South Carolina, USA. All values are expressed in ppm wet weight except ^{137}Cs, which are in Bq/g wet weight. A complete discussion of the bioaccumulation of these metals in various tissues of the same individuals used in this study can be found in Burger et al. 2002.

Metal	Site	*n*	Mean	Median	Minimum	Maximum
Se	Ash Basins	12	1.71	1.20	0.28	4.72
	Offsite	25	0.34	0.28	0.16	1.41
	Pond B	9	0.47	0.44	0.33	0.85
	Steel Creek	10	0.27	0.28	0.16	0.40
	Upper 3 Runs	12	0.24	0.21	0.09	0.46
Hg	Ash Basins	12	0.13	0.13	−0.06	0.36
	Offsite	25	0.05	0.07	−0.05	0.14
	Pond B	9	0.28	0.23	0.02	0.60
	Steel Creek	10	0.47	0.31	0.16	1.10
	Upper 3 Runs	12	0.44	0.32	0.10	1.07
As	Ash Basins	12	0.09	0.07	0.01	0.26
	Offsite	25	0.12	0.11	0.06	0.52
	Pond B	9	0.17	0.15	0.13	0.32
	Steel Creek	10	0.11	0.11	0.07	0.15
	Upper 3 Runs	12	0.11	0.10	0.07	0.16
Mn	Ash Basins	12	0.26	0.23	0.11	0.64
	Offsite	25	0.36	0.31	0.19	1.35
	Pond B	9	0.34	0.32	0.26	0.56
	Steel Creek	10	0.75	0.72	0.17	2.39
	Upper 3 Runs	12	0.24	0.23	0.13	0.40
Ni	Ash Basins	12	0.20	0.06	0.01	1.05
	Offsite	25	0.35	0.12	0.07	3.79
	Pond B	9	0.12	0.08	0.06	0.33
	Steel Creek	10	0.15	0.14	0.05	0.34
	Upper 3 Runs	12	0.11	0.08	0.04	0.34
Cu	Ash Basins	12	1.10	1.13	0.42	1.74
	Offsite	25	10.25	2.21	1.43	68.53
	Pond B	9	2.88	2.29	1.81	6.68
	Steel Creek	10	16.04	3.47	1.00	64.21
	Upper 3 Runs	12	5.66	1.41	0.69	52.78
Sr	Ash Basins	12	0.17	0.15	0.10	0.34
	Offsite	25	0.30	0.25	0.12	1.40
	Pond B	9	0.23	0.20	0.10	0.58
	Steel Creek	10	0.40	0.29	0.10	1.35
	Upper 3 Runs	12	0.19	0.19	0.12	0.27
Cr	Ash Basins	12	0.28	0.27	0.25	0.32
	Offsite	25	0.61	0.52	0.37	2.32
	Pond B	9	0.57	0.54	0.44	1.10
	Steel Creek	10	0.54	0.55	0.20	0.84
	Upper 3 Runs	12	0.32	0.33	0.13	0.48
Cd	Ash Basins	12	0.01	0.00	0.00	0.04
	Offsite	25	0.04	0.03	0.02	0.12
	Pond B	9	0.03	0.03	0.02	0.04
	Steel Creek	10	0.03	0.03	0.03	0.04
	Upper 3 Runs	12	0.04	0.04	0.03	0.09
Pb	Ash Basins	12	0.34	0.27	−0.03	1.01
	Offsite	25	0.07	0.05	0.02	0.25
	Pond B	9	0.61	0.05	0.04	5.02
	Steel Creek	10	5.07	0.10	0.04	49.56
	Upper 3 Runs	12	0.09	0.06	0.04	0.26
Cs-137	Pond B	8	0.38	0.33	0.17	0.85
	Steel Creek	10	0.14	0.15	0.04	0.27