

THE USE OF SMALL MAMMALS AS INDICATORS OF HEAVY METAL
BIOAVAILABILITY IN A CONTAMINATED RIPARIAN ZONE

by

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(Under the Direction of I. Lehr Brisbin, Jr.)

ABSTRACT

Elemental composition of kidney, liver, and muscle tissue from a common small mammal, the cotton mouse (*Peromyscus gossypinus*), was determined in an ecosystem contaminated with Ni and U at the Department of Energy's Savannah River Site, Aiken, SC. Independent factors, such as season, sex, trap site, and the interactions between these factors, were used in assessing metal burden. The use of stable isotopes allowed characterization of the trophic structure within the small mammal community. However, for the six species captured, no relationship was found between trophic level and metal contamination. Season was the only significant factor to play a significant role in metal contamination. In every case, specimens captured in the fall had higher tissue metal concentrations. Levels of U in the species we collected were similar in concentration to small mammals captured in studies further upstream while Ni levels were observed to be lower than those observed in the same studies.

INDEX WORDS: *Peromyscus gossypinus*, stable isotopes, trophic level, metal burden, Uranium, Nickel

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DEDICATION

I would like to dedicate this thesis to my wife Cin, who, much earlier than myself, understood the road which lay before us and in whose patience and perseverance I found an anchor when I was lost. To my parents, whose joy and overwhelming happiness for me I am most proud of. And to my brother, who is proof that confidence in oneself can not be obtained thru condition of employment.

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CHAPTER 1

INTRODUCTION

The widespread heavy metal contamination of soils and sediments at Department of Defense (DOD), Department of Energy (DOE), and related facilities is a costly legacy of the U.S. defense mission [1]. Recently, attention has been focused on the importance of trophic transfer in the spread of contamination in the environment. Studies have shown correlations between environmental risk of contamination and trophic positions within impacted ecosystems.

In the past, the majority of research dealing with trophic transfer and bioaccumulation was performed in aquatic ecosystems [2]. Examples such as Gilbertson [3] showed the transfer of contaminants through the water-plankton-fish-bird food chain, which had been responsible for the extirpation of certain bird species in the Great Lakes Basin. Recently however, studies began to also focus on the transfer of contaminants within the terrestrial environment and the risks posed to upper trophic level organisms. Connell & Markwell [4] observed that organic contaminants present in water, air, soil, and sediments move in a predictable fashion through invertebrates, fish, plants, mammals and birds, within the food web. They showed that concentrations of pollutants increased with trophic level, and thus posed a significant risk to consumers at upper levels (top carnivores).

When describing how contaminants behave in the environment, the terms bioconcentration, bioaccumulation, and biomagnification are widely used. However, many aspects of these processes are still debated, and currently unresolved [5,6]. A review of the literature indicates that the terms bioconcentration, bioaccumulation, and biomagnification are

often used inconsistently [6]. For the purposes of this research, the following definitions will apply to these terms: (1) *Bioconcentration* is the intake of a chemical contaminant by an organism and the subsequent concentration of that contaminant within the organism's tissues to a level that exceeds ambient environmental concentrations [5,6]. (2) *Bioaccumulation* is the process by which chemical contamination in an organism increases with each step in the food chain [5]. (3) *Biomagnification* is the process by which chemical contaminants are concentrated at levels that exceed chemical equilibrium, from dietary absorption of the chemical [5]. The bioconcentration, bioaccumulation, and biomagnification of chemical contaminants in a terrestrial environment are dynamic processes that involve many interconnected variables. For example, the potential for a chemical to bioconcentrate, bioaccumulate, or biomagnify in organisms and food webs may be dependent upon the properties of the chemical (i.e. its resistance to degradation), environmental factors (i.e. concentration of other chemicals and redox potential), biotic factors (i.e. The organism's trophic position and metabolism), and bioavailability (i.e. transport mechanisms and degree of contamination) [5-10]. This list of factors affecting these variables is not complete, and only serves to highlight how dynamic these processes truly are, which makes determining the ecological risks associated with the potential for bioaccumulation especially difficult.

Risk assessment evaluates the probabilities of negative consequences associated with specific contamination levels and then develops and organizes guidelines relevant to the proposed risks. Three important approaches to assessing environmental risk and environmental impact are: (1) food-chain analyses, (2) ecological endpoints, and (3) use of a target species. Food-chain analyses evaluate dietary sources (food and water) of toxicants, and can be used to evaluate risks associated with chemicals in the environment, especially those that bioaccumulate

in long term, low-level exposures. They are therefore a primary tool for evaluating risks to wild animals [11]. The identification of well-defined assessment endpoints is another critical step when conducting an ecological risk assessment. Assessment endpoints represent valued ecological entities and their attributes, upon which risk management actions are focused (i.e. small mammal species). By understanding the role of such endpoints, the potential for contaminant bioaccumulation and trophic transfer within the food web can be established [12]. An understanding of target species is also critical when assessing risk since contaminants may affect species differently. Variables such as age, sex, reproductive state, and physiological condition must be taken into consideration when assessing the risks posed by contaminants. Moreover, environmental risk assessment must not focus on chemical type alone, but also concentration, because both abundant and trace contaminants can cause toxic effects when exposure exceeds the critical threshold [13,14].

The use of laboratory mice to evaluate risks posed by various contaminants has been customary for decades, and toxicologists have long used these laboratory mammals as a surrogate species for man [15]. Only recently have efforts focused on wild mammals to understand the bioavailability and uptake of metals within a natural ecosystem. For example, small mammals may serve as intermediaries for the transfer of toxic metals to higher trophic levels [16]. Other studies [17-19] have also shown that small mammals can be an important link in the transfer of heavy metals within the food web. Hunter et al. [20] measured the trophic transfer of Cu and Cd to small mammals in a terrestrial environment and showed these metals to be highly mobile. Unusually high concentrations of metals have been reported in small mammals inhabiting areas affected by mining activities [21], metal-rich sewage sludge [22,23] and motor vehicle exhaust emission [24].

For decades, research on DOE lands has successfully used small mammals in basic ecological research [25-27]. Golley et al. [27] described a variety of small mammals that were captured on the DOE's Savannah River Site (SRS) located near Aiken, SC (the facility investigated in this study), from 1955 through 1973, focusing on habitat utilization and population structure. Such studies demonstrate the utility of these animals in studies of fate and transport of contaminants.

To understand the potential for contaminant uptake by free-living small mammals, their characteristics and life histories must be understood. Small mammals are often ideal for studying contaminant uptake because of their limited home ranges, reproductive capacity, usually omnivorous diet, and their frequent role as prey items for a variety of predators [28]. With this in mind, cotton mice (*Peromyscus gossypinus*) can serve as a particularly useful receptor species. There is considerable literature describing their life history [29-33] which allows for a better understanding of their potential to accumulate contaminants. Their home ranges have been well documented [34-36], which allows a better understanding of the relationship between site and exposure. *P. gossypinus* is also a good receptor species because of their population dynamics. As reported by Pournelle [33], their gestation period is 23-30 days, so an average of 3 litters per year can be expected in South Carolina, with an overall litter size ranging from 2-6 with a mean of 3.6. Lastly, Layne [37] reported an average life expectancy of 1.7 months in the wild with a maximum of ~ 5 months.

P. gossypinus is considered an opportunistic omnivore, [29,33,38]. While dietary habits can be helpful in studying the broad impacts of ecosystem contamination, an understanding of contaminant transfer in the lower ends of the food chain is essential. Hunter et al. [20]

demonstrated that the position in the food chain can directly affect the degree of exposure to contaminants.

While using a known omnivore as their target species, Hunter et al. [20] showed that although invertebrates were the most contaminated dietary component, providing 40 – 50% of cadmium intake, they only accounted for no more than 5% of the actual diet.

The effects of seasonal variation must also be understood when assessing the bioavailability of contaminants to consumers. Hunter et al. [20] observed that cadmium ingested by wood mice (*Apodemus sylvaticus*) showed seasonal variation, which was attributed to the availability of invertebrates.

Diet can be inferred by ratios of stable isotopes, which can help quantify the trophic position occupied by an animal [39]. There is considerable interest in using stable isotopes, particularly those of carbon and nitrogen, to evaluate the structure and dynamics of ecological communities [40-44]. There is little isotope fractionation as carbon is passed up a food chain, however, the ratio of $^{15}\text{N}/^{14}\text{N}$ increases by about 3‰ with each trophic level [45]. This is due to protein formation, degradation, and excretion of N. One advantage of stable isotopes is that they combine the benefits of trophic level analyses and the flow of elements such as C and N in food web analysis. That is, they facilitate estimates of energy or mass flow through ecological communities, and therefore can characterize the functional role of organisms within food webs [39]. Studies have demonstrated the use of stable isotope analysis to evaluate food web structure and material flow within a variety of systems [46-48]. These studies have shown that stable isotopes have the potential to simultaneously explain complex interactions, including omnivory, and to track (trophically) energy or mass flow.

Due to its previously studied life history and its abundance within each study site, *P. gossypinus* was chosen to be the indicator species used to measure the bioavailability of known heavy metals within our study site. Assessments of metal contamination were made in different seasons (spring vs. fall) in order to determine seasonal trends of metal burdens.

The purpose of this study was to determine heavy metal bioavailability within a disturbed riverine system located on the Savannah River Site and to document differences with season. Specific questions include: (1) is metal accumulation a function of $\delta^{15}\text{N}$ (a change in trophic level), site, and a higher order interaction term, or (2) is metal accumulation a function of $\delta^{15}\text{N}$ and site, and if so, does the $\delta^{15}\text{N}$ accumulate in the same way at every site, or (3) is metal accumulation a function of $\delta^{15}\text{N}$ and does not change between sites?

CHAPTER 2

MATERIALS AND METHODS

Study Site:

The Savannah River Site (SRS) is a 777-km² former nuclear production facility of the U.S. Department of Energy (DOE), located in west-central South Carolina. The site was closed to public access in 1952, and currently plays a key role in the nation's defense mission by housing nuclear materials and waste processing sites, as well as research laboratories (Figure 1). Roughly 80% of the site is forested and is managed primarily for commercial timber production (pine).

Throughout the years of processing, developing and storing of nuclear materials on the SRS, there have been consequential contaminant releases into the environment. One of the major areas of the SRS to be affected with such occurrences is the Steed Pond – Tims Branch (SP-TB) system (Figure 2). SP-TB is a second-order stream system located in the northwest part of the SRS [49] (Figure 2). It is an ideal location for investigating the biogeochemical cycling of heavy metals as it lies within one of the best-characterized environments and offers riparian and wetland habitats for study. The SP-TB system consists of Steed Pond (SP), Beaver Pond (BP), which is located downstream from Steed Pond, and still further downstream Pond 25, which is another former impoundment. The Tims Branch (TB) sampling site was located entirely with this Pond 25 area downstream from BP. Whipple et al. [50] described the SP-TB system as: 1) bottomland hardwood forest including numerous beaver ponds; 2) the floodplain of Steed Pond, which exhibits early successional growth; and 3) Tims Branch, which displays mature growth at

an advanced successional stage. Beginning in 1954, two industrial complexes, the A and M Areas of the SRS, began production of aluminum-clad U targets. Along with large quantities of depleted and natural U, Ni and Al also entered and consequently contaminated the nearby SP-TB riparian ecosystem. In addition, smaller amounts of Cu, Cr, Zn, Pb and other metals, associated with the production of defense-related nuclear fuel and targets, were also released into this system. Effluent discharge to SP-TB ceased in 1982, and in 1989 the M-area settling basin was removed from service, stabilized, and capped [51].

SP-TB is a small second-order black water stream system typical of the southeastern U.S., with a sandy bottom and an organically rich sediment zone. During processing, heavy metals were discharged into the SP-TB system. They then collected within SP, which served as a settling basin for the collection of contaminants between the mid-1950's and 1985 [52].

Approximately 44,000 kg of depleted U were released into SP, [49] accounting for nearly 97% percent of the total gross α -activity released by the SRS, with 61% of the activity being released between 1966 and 1968 [52]. Associated wastes such as sulfuric, phosphoric, and nitric acids were also discharged into this stream.

It has been estimated that greater than 70% of the released uranium and other heavy metals were retained within the sediments of SP [49,52]. A 1984 breach in the man-made dam at SP released its contaminated sediments further downstream. Since the breach, detailed studies on SP have shown selective erosion of localized, unvegetated areas. These unvegetated areas are "hot spots" characterized by elevated concentrations of metals and radionuclides, which precluded plant establishment in some areas following dam failure [53].

When the 1984 breach occurred at SP, another pond, Pond 25 (in TB), filled downstream and, like SP, became a settling basin for contaminants from A and M areas (Figure 2). As with

the dam at SP, soon after the formation of Pond 25 within the settling basin TB, it too was breached allowing contaminants to further flow into the Upper Three Runs System. Since then, elevated levels of heavy metals (U, Ni, Cu, Zn, and Pb) have continued to be found in sediments and biota within TB [54].

Further surveys of the surrounding terrain have shown that the lateral spread of contamination from SP-TB has been constrained by stream morphology [52] and that most of the contaminant inventory has been deposited in the corridor's natural and man-made impoundments such as beaver ponds and former farm ponds. The BP site, which is an actively-used beaver pond located upstream from TB, (Figure 2) has acted as a natural settling basin between the two locations (SP and TB). Samples were collected to survey heavy metal contamination in the small mammal community inhabiting the area of the wetland vegetation bordering the BP.

As a control for the SP-TB contaminated sites, small mammals were also collected from another similar riparian habitat, Boggy Gut (BG), also located on the SRS. This site included geomorphologic features and general habitat characteristics similar to those of the TB area [1] (Figure 2).

Mammal Collections:

Tims Branch:

Four separate and parallel transects were established at 23m intervals covering the TB study site as described in previous literature [12]. These transects paralleled a stream which currently flows thru the TB study site. Each of the 4 transects was oriented at 346° relative to N, and contained 19 trap sites at 10 meter intervals per transect except for the 4th line which only contained 18 trap sites due to the morphology of the basin (total = 75 trap sites). Every trap site along each transect contained a large Sherman live trap (7.62 x 8.89 x 22.86 cm) while every

other trap site also included a medium Sherman live trap (5.08 x 6.35 x 16.51 cm). This made it possible to catch a variety of small mammal species to determine the community structure.

Beaver Pond:

At the Beaver Pond trap site, only 3 equal and parallel transects could be established along the shoreline of the beaver pond. Each transect contained 13 trap sites, except for the 3rd transect which only contained 12 sites due to landscape restraints. As with the TB site, trap sites were 10 meters apart with transects 23 meters apart. Each trap site again, contained 1 large Sherman trap with an additional medium Sherman live trap at every other site. Each transect followed the shoreline of the active beaver pond and could not be set on an exact compass bearing.

Boggy Gut:

The sampling regime for this control site was the same as for the other two study sites, with 4 separate and parallel transects. Each transect was oriented at 96° relative to N, and contained 19 trap sites except for the 2nd transect which contained only 18 sites due to landscape constraints, totaling 75 trap sites. As with the contaminated locations, each trap site contained a large Sherman live trap while every other trap site also contained a medium Sherman trap. Trap sites were located at 10 meter intervals and transects were 23 apart. As with TB, BG also followed a steadily flowing stream, which ran through the study site.

While the main focus of this collection was *P. gossypinus*, every mammal trapped was collected, identified and tissue and organ samples taken. Trapping sessions were classified as spring (second week in April - second week in June) and fall (second week in September - second week in November). Individual trapping sessions at each site were continued until 20 *P. gossypinus* were captured or 3 consecutive weeks passed with no further *P. gossypinus* captured.

Each session usually averaged 6-8 weeks. Due to the stable isotopic approach used in this study, no pre-baiting was undertaken. Each trap was checked daily and animals were returned to the laboratory alive. Individuals were sacrificed within 24 hours of capture by quickly restricting air flow to the lungs; both kidney's, the entire liver, and muscle tissue from both quadriceps femoris were extracted, by hand, from each specimen. Each individual organ and tissue was placed into a Corning 2 ml polypropylene cryogenic vial, labeled and frozen in a REVCO Scientific upright commercial refrigerator/freezer at -70°C for later analyses of stable isotopes and heavy metals. Whole bodies were labeled and were also stored in a walk-in freezer (International Cold Storage walk-in freezer) for future studies. All small mammals captured in this study were handled according to animal care guidelines set forth by the University of Georgia (IACUC # A2003-10040-0), and the state of South Carolina scientific collecting permit issued to the Savannah River Ecology Laboratory.

Metal and Stable Isotope Analyses:

All liver, kidney and muscle tissue samples were freeze-dried prior to microwave digestion. Samples were weighed, placed in a LABCONCO freeze-drier and reweighed until organ and tissue moisture weights stabilized (≈ 7 days). The samples were then removed from the freeze-drier and reweighed one last time to determine percent moisture.

Freeze-dried, tissue/organ samples were digested, using 2.5 ml of trace-metal grade (Fisher Scientific) HNO_3 and placed in a Teflon microwave digestion vessel. The vessel was then capped and digested in a CEM MDS-2000 microwave using a variable powered program with increasing microwave power applied over a 45-minute program. After cooling, the vessels were uncapped and 1 ml of 30% H_2O_2 was placed in each vessel. The vessels were then recapped and placed into the microwave for a repeat of the previous microwave procedure. After

cooling, each digestion was brought up to a volume of 25 ml using a Quantum IX Millipore filtration device in volumetric flasks. All laboratory equipment and containers were washed in 10% HNO₃ prior to use. Each digestion set was composed of 12 separate vessels, each of which included a duplicate set of samples, a blank, and a standard reference (DORM-2/DOLT-2, NRC-CNRC, Ottawa, Canada). Digested samples were analyzed for Al, Cr, Co, Ni, Cu, Se, Mn, Cd, Pb, U, and Hg by an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a Perkin Elmer SCIEX (ELAN DRC plus) in standard operating mode. The procedure followed methodology outlined in EPA method 6020 and quality control procedures were based on EPA procedure SW-846.

Detection Limits:

Samples below detection limits (BDL) were encountered when analyzing for heavy metal concentrations, based on method detection limits calculated as 3 times the standard deviation from the blank samples plus the average value for the blank samples. The BDL's (in ppm) were: Al = 0.0301, Ni = 0.0141, Cu = 0.0091, Se = 0.0043, Mn = 0.0039, Cd = 0.0014, U = 0.00003, Hg = 0.0039. Samples registering as BDL were entered as the detection limit itself in all statistical calculations. Percentages of samples registering as BDL are presented in (Table 3) for all metals analyzed.

Elemental analysis-isotope ratio mass spectrometry was employed to measure the total nitrogen and carbon content, and quantify ¹⁵N/¹⁴N and ¹³C/¹²C ratios of each muscle sample from each species. Prior to isotopic analyses, samples were homogenized by cryogenically grinding them with a Spex/Certiprep 6750 freezer/mill. Approximately 2 mg of ground sample were placed in individual pre-cleaned tin capsules and weighed to ± 1.0 µg using an ultramicrobalance. Capsules were then sealed and placed in the auto sampler of a Carlo Erba

Elemental Analyzer (NA1500) attached to a continuous flow isotope ratio mass spectrometer [Finnigan Delta +XL (Finnigan-MAT, San Jose, CA)]. Samples were combusted to N₂ and CO₂ in oxidative-reduction furnaces, separated by gas chromatography, and then measured for ¹⁵N/¹⁴N and ¹³C/¹²C ratios on a mass spectrometer. A N₂(g) working standard was admitted prior to each sample combustion for calibration to an international AIR standard [55]. Stable isotope ratios were reported in per mil units (‰) using standard delta (δ) notation [56]. External working standards of bovine liver and acetanilide were analyzed to determine external precision; these standards were reproducible to better than ± 0.15‰ (1σ) for both δ¹⁵N and δ¹³C.

Potential food items for *P. gossypinus* were collected each season for ¹⁵N/¹⁴N and ¹³C/¹²C analysis. Grasses, shrubs, leaves and shoots (*Juncus sp.*, *Rubus sp.*, *V. rotundifolia*, and *Andropogon sp.*) which compose one-third of their diet [27,57] were collected during both the summer and fall trapping seasons, while seeds and nuts (*Quercus sp.*) were collected during the winter and spring trapping seasons. However *P. gossypinus* is an opportunistic omnivore [29,33,38] with nearly 68% of their diet composed of animal matter such as beetles, lepidoptera, and spiders, with snails being the most abundant species consumed [38]. These however, were not collected, although invertebrate data quantifying ¹⁵N and ¹³C values will soon be available (O'Quinn in review).

Food items were labeled according to the year and season and then freeze-dried using the LABCONCO freeze dry system. Once the drying had been achieved, the samples were combined, and homogenized by cryogenically grinding with a Spex/Certiprep 6750 freezer/mill. Approximately 5 mg of ground sample were placed into individual pre-cleaned tin capsules and weighed to ± 1.0 μg using an ultramicrobalance. Capsules were then sealed and placed in the auto sampler of a Carlo Erba Elemental Analyzer (NA1500) attached to a continuous flow

isotope ratio mass spectrometer [Finnigan Delta +XL (Finnigan-MAT, San Jose, CA)] and the same procedure was performed as for the muscle tissue.

Statistical Analyses:

Metal and isotope distributions were tested for normality using the Shapiro-Wilk statistic (PROC UNIVARIATE, version 8.1; SAS Institute). Hypotheses that data were sampled from normal distributions were rejected, and stem-and-leaf plots suggested log-transformation of data prior to analysis. Log transformation normalized all variables.

Analysis of variance models (ANOVA; PROC GLM; SAS Institute) were used to examine the relationship between metal burdens in kidney, liver, and muscle to location of collections, season, sex, ^{15}N values and their interactions. If the interactions were not significant, they were dropped from the model. For all tested models, Type III (partial) sums of squares and associated F -statistics were interpreted and least-squares means procedures were used to: (1) provide estimates of dependent variables that were adjusted for all effects in the models, and (2) provide mean separation tests. All statistical tests were considered significant at the $p \leq 0.05$ level. Means and standard errors were presented as back-transformed values of log least-squares means estimates (i.e., geometric means). When BDL's were encountered, the detection limit for that particular analysis was entered for that sample.

CHAPTER 3

RESULTS

Species Captured:

The most abundant species captured was the cotton mouse, *P. gossypinus* (n = 166). Other species captured included: short-tailed shrew, (*Blarina brevicauda*; n = 11), marsh rice rat, (*Oryzomys palustris*; n = 6), golden mouse, (*Ochrotomys nuttalli*; n = 5), and western woodrat, (*Neotoma floridana*) and hispid cotton rat, (*Sigmodon hispidus*) with n = 4 each. These species are representative of those found in other studies conducted at the DOE's Savannah River Site [28,58,59].

Below Detection Limits:

Samples below detection limits were encountered when analyzing for heavy metals, particularly with U. Percentages of BDL's for all tissue/organ samples were calculated for each metal (Table 3). Due to the number of samples below detection limits, results for U should be interpreted with caution.

Metal Tissue Concentrations:

General Linear Model (GLM) models were run with ¹⁵N as a covariate; and showed no statistical significance and it was therefore not included in the following analyses. The following results were taken from statistical models performed on *P. gossypinus* only. In the case of U,

statistical tests were performed, but due to the high frequency of concentrations of samples below detection limits, interpretation should be viewed with caution.

Kidney:

Metals in kidney varied with site, sex, season and their interactions (Table 1). Al, Cd, and U concentrations differed by location, while Ni and Se approached statistical significance (Table 1). Bonferroni comparisons showed that Al in kidneys from animals at the BG control site, was significantly higher than in those from both contaminated sites which did not differ from each other (Table 2). This relationship was similar to that of Ni, in which BG kidneys were also significantly higher in Al than those from TB. However those from BP were also significantly higher than TB and did not differ than BG. Moreover, BG kidneys were significantly higher in Cd than those from BP, but did not differ from those from TB. Se concentrations did not differ between BG and TB; however kidney tissues from BP were significantly higher than those from both of these the other locations. U showed higher concentrations at the TB site than at the other two locations, which did not differ from each other. There were no significant differences between any of the 3 sites for Cu, Mn, or Hg.

In only 1 case (Cu) was sex significant in the GLM (Table 1), while Mn and Cd approached statistical significance. In each of these three cases males had higher concentrations than females (Table 1). Sexes did not differ for Al, Ni, Se, U, and Hg.

For all metals observed within the kidney, there was a statistical significance between metal concentration and season with levels showing an increase in the fall vs. spring (Table 1, Figure 3). There was a significant interaction between site x season for Al and Ni, but no significant interaction for sex x season for any metal (Table 1).

Liver:

As with kidney, metals in liver differed between locations of collections, sexes, seasons and their interactions (Table 1). Al, Cu, Se, Mn, Cd, concentrations differed significantly between locations (Table 1). Bonferroni tests showed that U and Al were significantly higher at TB than at either BP or BG, the control site, but the latter two did not differ from each other (Table 2). BG and TB were significantly higher in Se, Mn, and Cd than BP, while not differing from each other, while Cu was the only metal in which liver concentrations from BG were higher than at either of the other sites (Table 2). In this case, liver at TB did not differ from BP. There were no significant differences between any of the 3 sites for either Ni or Hg (Table 2).

Only Mn differed significantly between the sexes (Table 1), with concentrations in the livers of males exceeding those of females. There were no significant difference between the sexes for liver Al, Ni, Cu, Se, Cd, U, and Hg. As in the case of kidney, there was a significant effect of season with liver concentrations also showing an increase in fall vs. spring (Table 1, Figure 4). There was a significant interaction for location x season for Al, Ni, and Cu, and there was a significant interaction for sex x season for Ni (Table 1).

Muscle:

As with kidney and liver, metals in the muscle tissues behaved differently in their relationships with location, sex, season and their interactions (Table 1). Only Cd and U differed between locations in muscle tissues (Table 1). Bonferroni tests showed that Cd levels at BP were significantly higher than at both TB and BG which did not differ (Table 2). There were no significant differences between any of the 3 sites for Al, Ni, Cu, Se, Mn, and Hg (Table 2)

In only 1 case (U) did the sexes differ in muscle metal concentrations with those of males being higher (Table 1). As with the kidney and liver, all metal concentrations in muscle, showed

a significant effect of season on muscle metal levels with concentrations again showing an increase in fall vs. spring (Table 1, Figure 5). There was a significant interaction effect for location x season for Al, Ni, Cu, U and Hg and there was also a significant interaction between sex x season for Se (Table 1).

Distribution of Metal Concentrations between Organ/Tissue Types:

Tims Branch:

Kidney, liver, and muscle tissues showed differences in the patterns of their relative metal concentrations (Table 2). Bonferroni tests showed that kidney and liver concentrations for Al, Ni, Cu, and Se were similar. However, muscle was significantly different (sometimes higher, sometimes lower) from the other tissues for these metals (Table 2). Furthermore, concentrations of Mn and Cd were significantly different in all three tissues. Hg showed significant difference in metal concentrations between the kidney and liver but showed no difference in metal concentrations within the muscle (Table 2). In the cases of Cu, Se, Cd, and Hg the highest concentrations were observed in the kidney. Ni and Al levels were observed to have the highest concentrations in the muscle and Mn levels were observed to have the highest concentrations in the liver (Table 2)

Beaver Pond:

At this location, metal concentrations in kidney and liver for Cu, Mn, and Cd, were not significantly different, but the tissues were always higher than that of muscle (Table 2). Se and Hg concentrations found in the liver, kidney and muscle did not differ significantly in concentration levels, while Al concentration levels differed significantly across liver, kidney, and muscle. Ni concentrations were not significantly different between the kidney and muscle, but they were significantly lower in liver (Table 2). In the cases of Se, Cd, and Hg, the highest

concentrations were observed in the kidney, with the highest concentrations of Cu and Mn in the liver and the highest concentrations of Cu and Mn levels in the muscle (Table 2).

Boggy Gut:

At this location, Al, Mn, and Cd, differed between kidney, liver, and muscle while Cu and Se levels did not differ between kidney and liver, but were lower in muscle. Hg showed no differences between liver and muscle concentrations, but kidney levels were higher. There was no significant difference in Ni levels in kidney and muscle, but concentrations were lower in liver (Table 2). In the cases of Se, Cd, and Hg, the highest concentrations were observed in the kidney, with the highest concentrations of Cu and Mn in the liver and the highest concentrations of Al and Ni levels in the muscle (Table 2).

Stable Isotopic Analyses of Metal Contamination:

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

Stable isotope data were collected from muscle samples from all species that were found at all 3 locations. The range of $\delta^{15}\text{N}$ values for muscle, for all species captured at all three sites, was relatively large (2.78 to 7.61), indicating that these animals fed over a range of 2-3 different trophic levels (Figure 6). While there were wide ranges of $\delta^{15}\text{N}$ values within species studied, there was no significant seasonal difference in $\delta^{15}\text{N}$ values at the $p \leq 0.05$ level between the fall and the spring for *P. gossypinus* (Figure 7).

Mean values of $\delta^{13}\text{C}$ were relatively similar, ($\pm 1 \sigma$), between species: *O. nuttalli* (-24.73), *P. gossypinus* (-24.36), *N. floridana* (-25.34), *S. hispidus* (-25.72), *B. brevicauda* (-23.24), and *O. palustris* (-25.47), indicating that these species were all consuming similar terrestrial C_3 plants (Figure 8).

The $\delta^{15}\text{N}$ values for food items of the *P. gossypinus* collected during the summer months averaged 1.59‰, while $\delta^{15}\text{N}$ values for food items collected during the winter months averaged 0.079‰ (Figure 9). The $\delta^{13}\text{C}$ values for these food items collected during the summer months averaged -29.66‰, while the $\delta^{13}\text{C}$ values for food items collected during the winter months averaged -29.95‰.

CHAPTER 4

DISCUSSION

Small mammals are common links in the transfer of contaminants from soils and vegetation to higher trophic levels. Thus, metal concentrations in small mammals can provide information regarding potential for bioaccumulation, within the ecosystem and food chain transport of these contaminants [21]. Stable isotope analysis of small mammal tissues can provide important information on these processes because, when properly applied, this technique can produce estimates of trophic position within complex trophic webs of ecological communities [45]. Hunter et al. [20,60,61] examined the ecotoxicology of metals in a contaminated grassland ecosystem, specifically the food-chain relationship of copper and cadmium in 3 different mammals: common shrew (*Sorex araneus*), field vole (*Microtus agrestis*) and wood mouse (*Apodemus sylvaticus*) representing 3 different specialized diets, insectivore, herbivore, and omnivore, respectively. The study found that metal contamination levels in small mammal diets followed the order *S. araneus* > *M. agrestis* > *A. sylvaticus*. The study concluded that *S. araneus*, which fed primarily at a higher trophic level compared to the other two species, was the only species to show metal accumulation at each of their study sites.

Of the six species studied here, five species are generally considered omnivores, with *B. brevicauda* having the highest $\delta^{15}\text{N}$ values, which is consistent with the known foraging ecology of a carnivore/insectivore [62]. The five remaining species were omnivores; however each species had a slight, but non-significant, difference in mixtures of $\delta^{15}\text{N}$ enrichment, suggesting

the consumption of different food resources. *O. nuttalli* was the least enriched in ^{15}N values, indicating that invertebrates and other trophically higher organisms were not consumed. *P. gossypinus*, *N. floridana*, and *S. hispidus* tended to have diets more enriched in ^{15}N , although differences among these species were not significant. *O. palustris*, one of the four omnivores, and *B. brevicauda*, a strict carnivore, were the most enriched in ^{15}N and did not differ from each other in concentration levels, indicating that these species were feeding at slightly higher trophic levels than the others. This is supported by previous studies showing that *O. palustris* and *B. brevicauda* tend to eat a high proportion of animal material [62,63]. Since sample sizes were very low for all species, except *P. gossypinus*, any conclusions concerning the relationship between ^{15}N and factors such as metal concentration, sex, and season would be highly speculative at the community level. *P. gossypinus*, despite a large range in ^{15}N values, did not show a significant seasonal shift in diet. However, there was a slight shift in $\delta^{15}\text{N}$ values for food samples collected in the spring vs. the fall. This is consistent with studies showing the diet of *P. gossypinus* to be composed primarily of animal material during the spring/summer [38]. This increase in $\delta^{15}\text{N}$ values for food items is reflected in the $\delta^{15}\text{N}$ values seen in *P. gossypinus* muscle in fall vs. the spring; however this was not statistically significant. As will be discussed below, metal accumulation in small mammals was also higher during the fall than the spring.

The lack of statistical significance in the seasonal shift of $\delta^{15}\text{N}$ values, in contrast to the significance of the effect of season on metal concentrations observed within *P. gossypinus*, resulted in the lack of any demonstrable relationship between $\delta^{15}\text{N}$ and metal burden in this study. This could be due to the fact that these metals do not biomagnify in rodents at the time scale that was investigated. Alternatively, a number of factors could have potentially confounded the relationship between $\delta^{15}\text{N}$ and metal concentrations in muscle tissue. Discrete

relationships between body size, nutritional stress, metabolism and physiology, age and gestation period could not be characterized in this study. All may influence the enrichment $\delta^{15}\text{N}$ in muscle tissue [64-71].

Although Gaines et al. [72] showed a direct relationship between metal concentration and $\delta^{15}\text{N}$ values within raccoons (*Procyon lotor*) on the SRS, this relationship was not found in this study with *P. gossypinus*. A possible factor that may contribute to these differences could be that in the raccoon study, the animals foraged over a much larger spatial scale. That is, dietary shifts were not seasonal, but rather were dependent upon location and/or differences in food availability. In that study, the range of $\delta^{15}\text{N}$ values for *P. lotor* muscle tissue was relatively high (4.28 to 13.68‰) which was similar to the range of $\delta^{15}\text{N}$ values found here for muscle tissue from *P. gossypinus* (0.73 to 10.69‰). Not only was there a large range in $\delta^{15}\text{N}$ values for *P. gossypinus*, but for the entire community of small mammals as well. However, sample sizes, which were low except for *P. gossypinus*, prevented further investigation of this relationship. Future studies focusing on controlling for these factors (sample size, seasonal variation or food variability) should help improve the understanding of food web structure and contaminant transfer in this environment.

The $\delta^{13}\text{C}$ values for *P. gossypinus*, with outliers taken into consideration, had ranges that were very small (-22.64 to -26.05‰) for TB species alone. For the entire study the values for all small mammals still only ranged from -22.64 to -26.91‰, indicating that all species were consuming a similar proportion of C_3 plants (or consumers derived from them).

P. gossypinus was the most abundant species at each study site and therefore was used as the focal species for the investigation of tissue concentrations (Appendix 1). Metal concentrations in kidney, liver, and muscle from *P. gossypinus* differed between locations, with

some significant differences found between organs. When the relationship between metal burden and tissue type was investigated in this species, there were several cases where metals differed between locations within tissues. This was not surprising considering the diversity of both contaminants and landscape structure of the impacted riparian zone. The BP and TB sites were morphologically dissimilar and were different distances from the source term. While the TB site was a bottomland hardwood forest, displaying mature growth at an advanced successional stage, the BP site had a dynamic shoreline with early successional growth. These natural features make the potential impact of the source term (in our case from SP) very different. Since the TB site was contaminated via a settling basin (farm pond) that no longer exists, the soil in this bottomland community allowed contaminants to move directly to consumers through soil ingestion and accumulation in food items. Conversely, most all of the contaminants at the BP site were still within the sediments and wetland plant communities (i.e. mostly underwater) in the still active pond basin (Gaines, Punshon, and Murray unpublished). This would limit their movement to terrestrial organisms that utilize only the pond margin and immediate surrounding area. *P. gossypinus* trapped in the wet / flooded areas of the pond, were likely to consume animal and plant material derived from aquatic systems in the adjacent pond. These different conditions, the drying up and therefore oxidation of the soils at the TB basin vs. continuing flooding/drying events at the BP pond margin undoubtedly had an effect on the vegetation from a physical and chemical standpoint. The control site, BG, which surrounded a flowing stream, was similar to the bottomland hardwood forest representative of the TB site, in that the soil again allowed for the movement of elements directly to consumers through soil ingestion as well as from accumulation in food items. This “control” site was also subject to

anthropogenic influences from agricultural and residential inputs upstream and thus was also exposed to potential accumulation of trace elements.

It was assumed that most of the small mammals that were captured within each study site spent most of their time at that location. Estimates of density of *P. gossypinus* on the SRS range from 3.3 to 8.7 individuals per ha [26]. This is supported by other studies [35,73] that determined *P. gossypinus* home range to fall between 0.45 ha and 0.49 ha, which was well within the limits of our trapping grids. Home ranges are important in density estimates due to the restrictions imposed by our study sites. However, there is always the potential to capture individuals whose home ranges only marginally included the contaminated study site or individuals that may have been dispersing. Differences in metal levels of small mammals between study sites could also be due to differences in the potential to ingest contaminated soil. Studies have found that small mammals with similar foraging habits to *P. gossypinus*, exhibited digging as part of their foraging techniques [74], accumulating as much as 2% of their diet of soils [75]. The soils in the TB system were more contaminated than those found at the trapping grid of the BP study site which could have made contaminants more easily available to these small mammals. Punshon [54] found that contaminants were resuspended on to standing vegetation in TB thus providing another source of contaminant accumulation in primary consumers.

Whenever sex was a significant variable influencing metal concentrations in rodent tissues, males were observed to always accumulate higher metal concentrations than females. This could have been due to physiological and developmental differences between the sexes: females have the potential to transfer some of their metal burdens to their young. Further, a different metabolic rate between the sexes may result in different absorption rates of metal

concentrations. Moreover, trapping studies have shown a sex ratio favoring males [33,37,76], which may be due to the fact that they are more likely to forage over larger areas for food. This increased foraging area, could result in an increase in exposure to potential toxicants.

Season exerted the most significant effect on metal concentration in rodent tissues in this study. For each organ/tissue studied, higher concentrations of metals were found in the fall, indicating that studies focusing on contaminant mobility within a food web involving small mammals such as these should consider seasonality. The feeding behavior and physiology of the small mammal community is most likely the explanation for these differences. Shifts in both diet and metabolism could influence the accumulation of these metals, and $\delta^{15}\text{N}$ data did show a tendency to shift according to season. Hunter et al. [20] also showed seasonal variation in small mammal metal concentrations, also indicating higher concentrations in the fall, through detailed dietary analyses for 3 separate species inhabiting contaminated and control grasslands.

Behavioral responses (i.e. movements between microhabitats) could also explain why toxicant accumulation was greater in the fall. Specifically, *P. gossypinus*, can exploit the protective vegetative canopy which exists throughout the summer. The canopy density, coupled with the increased length of daylight, allows small mammals to not only forage for longer amounts of time, but to forage more often than they would normally. This increase in exposure time to metals from the summer season would logically be expressed in higher concentration levels being observed in the fall season due to the turnover rates of toxicants in the tissues. The seasonal differences in metal concentration could also have been due to food availability. Calhoun [38] has shown that the diet of *P. gossypinus* consists of about 68% animal matter during the summer, including beetles, lepidoptera, spiders, and snails, while they tend to feed on seeds, nuts and fungus, which may be high in trace elements, during the winter. Another factor

that may have contributed to the seasonal shift in metal burdens could have been reproductive effort. Studies have shown [77] that there is a decline in reproductive activities during the summer months preventing females from depurating contaminants into their young. This also allows more time for foraging, which increases the exposure time to potential contaminants. Future studies designed to show seasonal differences between the sexes could explain reproductive factors effecting metal burden.

Other studies have focused on the internal interactions within small mammals, describing the different interactions of metal ions during absorption [78,79] while other studies have involved trace elements and proteins [80-83]. Chemical interactions found within the target species, coupled with environmental parameters (i.e. home range and density) could help explain why some species may be more susceptible to the uptake of some metals than others.

The retention and bioavailability of ingested metals are influenced by many physiochemical parameters. When metal concentrations were compared among tissue types (i.e. liver, kidney, muscle; Table 2), observations from all three sites revealed no regular pattern. This was somewhat surprising since studies have shown that both the liver and kidney readily accumulate higher levels of metal contaminants, and provide a “sink” for such contaminants [84-88]. This lack of any clear pattern of organ uptake may be due to biochemical functions that may be metal dependent. The lack of a consistent pattern in the present study may also be due to the differential interactions of available contaminants within the soil matrix as well as within the body.

The metal concentrations found in this study were consistently lower than those found in another small mammal study performed at SP, the upstream source term of contamination in the TB ecosystem. Punshon et al. [12] found that both *S. hispidus* and *O. palustris* accumulated

measurable levels of both U and Ni, the two contaminants of concern in the TB ecosystem. The mean amount of Ni found in both liver and muscle tissue in *S. hispidus* and *O. palustris* from that study were an order of magnitude higher than the maximum values of Ni found in the same tissues for *P. gossypinus* from our study site located downstream. Even though the species sampled at the SP site were different than the ones we sampled, stable isotope data presented here suggests that they likely occupy consuming at the same trophic level as *P. gossypinus*, with a diet of a mixture of grasses and primary consumers mainly invertebrates, which is consistent with previous literature [38,63,89-93]. Mean amounts of U, however, were similar in small mammal tissues from both studies.

A critical question concerning the TB basin was bioavailability of heavy metals to a small mammal community. It is likely that contaminants were transported in TB during periods of elevated stream flow and accumulated in the sediments of the basin, leaving stream sediments deposited on the surface of the soils along the TB corridor. Studies have shown that the physical mobilization of contaminated wetland sediments from SP has contributed to high levels of Ni and U in the soil and vegetation of TB [54]. However, small mammal data from this present study suggests that Ni at TB diminished in bioavailability from levels observed at the source term (SP). Although U was found to be in very low concentrations in small mammals at TB, suggesting a negligible bioavailability, its bioavailability was consistent with levels reported by other studies performed upstream- indicating no decrease in bioavailability of U concentrations to small mammals.

Studies have shown the importance of small mammals as indicators for the bioavailability of contaminants, but that is just one aspect of assessing the risk posed by contaminants. Understanding how the general ecology of the indicator species (i.e. the species' behavior,

feeding and breeding habits) could have an effect on contaminant concentrations is vital to risk assessment. Other factors such as contaminant mobility is also important as Marquenie [94] suggests, when measuring the bioavailability of metals, it is important to keep in mind that a given concentration of toxicant that may be harmful in one soil or sediment type, may have no effect in another due to variations in metal speciation and thus environmental availability. Small mammals are important when addressing risk to the environment, but they are only one piece of the puzzle that must be understood if a comprehensive assessment is to be made.

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Table 1. General Linear Model results (F -values shown with P -values in parenthesis) showing the effects of location, sex, season, and their interactions on metal tissue concentrations of cotton mice (*Peromyscus gossypinus*). If an interaction term was significant, the independent variables were kept in the model regardless of P -value. Model results are reported using Type III sums of squares. Non-significant variables are denoted as NS. * Test could not be performed due to the frequency of tests that fell below detection limits. Means and standard deviations should be interpreted with caution.

KIDNEY					
Metals	Site	Sex	Season	Site x Season	Sex x Season
Al	2.96 (0.0545)	NS	6.17 (0.0141)	7.69 (0.0007)	NS
Ni	2.85 (0.0606)	NS	31.92 (<0.0001)	4.54 (0.0122)	NS
Cu	NS	3.78 (0.0537)	81.03 (<0.0001)	NS	NS
Se	2.66 (0.0734)	NS	45.52 (<0.0001)	NS	NS
Mn	NS	3.54 (0.0618)	44.76 (<0.0001)	NS	NS
Cd	13.69 (<0.0001)	3.25 (0.0734)	42.72 (<0.0001)	NS	NS
* U	20.65 (<0.0001)	NS	56.17 (<0.0001)	NS	NS
Hg	NS	NS	8.13 (0.0049)	NS	NS
LIVER					
Metals	Site	Sex	Season	Site x Season	Sex x Season
Al	6.36 (0.0022)	NS	70.61 (<0.0001)	8.14 (0.0004)	NS
Ni	1.26 (0.2861)	0.51 (0.4748)	18.77 (<0.0001)	4.04 (0.0195)	4.16 (0.0431)
Cu	3.66 (0.0279)	NS	3.91 (0.0497)	4.17 (0.0173)	NS
Se	5.32 (0.0058)	NS	29.12 (<0.0001)	NS	NS
Mn	5.32 (0.0058)	3.96 (0.0483)	15.52 (0.0001)	NS	NS
Cd	14.61 (<0.0001)	NS	33.87 (<0.0001)	NS	NS
* U	5.52 (0.0048)	NS	73.48 (<0.0001)	NS	NS
Hg	NS	NS	19.48 (<0.0001)	NS	NS
MUSCLE					
Metals	Site	Sex	Season	Site x Season	Sex x Season
Al	0.45 (0.6410)	NS	2.32 (0.1297)	4.66 (.0108)	NS
Ni	1.90 (0.1531)	NS	11.34 (0.0010)	5.50 (0.0049)	NS
Cu	1.51 (0.2236)	NS	146.21 (<0.0001)	3.92 (0.0217)	NS
Se	NS	1.99 (0.1605)	39.03 (<0.0001)	NS	2.92 (0.0894)
Mn	NS	NS	52.98 (<0.0001)	NS	NS
Cd	6.07 (0.0029)	NS	20.91 (<0.0001)	NS	NS
* U	11.20 (<0.0001)	4.43 (0.0370)	23.83 (<0.0001)	4.88 (0.0088)	NS
Hg	2.22 (0.1123)	NS	8.14 (0.0049)	2.65 (0.0738)	NS

Table 2. Effect of tissue type on metal concentrations in *Peromyscus gossypinus* for three trapping locations on the Department of Energy’s Savannah River Site, South Carolina, USA. Bonferroni multiple comparison tests were used to determine which tissues differed from each other. Means with the same letter are not significantly different. Geometric Mean and SE (back transformed) $P \leq 0.05$.

TIMS BRANCH	KIDNEY	LIVER	MUSCLE	F-value (P- value)
METALS				
Al	0.783 (1.501) A	1.592 (1.341) A	9.924 (1.069) B	20.56 (<0.0001)
Ni	0.016 (1.578) A	0.025 (1.513) A	0.190 (1.092) B	13.39 (<0.0001)
Cu	11.657 (1.074) A	11.202 (1.072) A	3.880 (1.075) B	75.83 (<0.0001)
Se	3.250 (1.160) A	2.850 (1.131) A	0.433 (1.296) B	36.33 (<0.0001)
Mn	3.097 (1.075) A	4.741 (1.076) B	0.817 (1.079) C	153.09 (<0.0001)
Cd	0.518 (1.289) A	0.145 (1.138) B	0.0001 (1.578) C	189.48 (<0.0001)
Hg	0.059 (2.034) A	0.0004 (3.426) B	0.005 (1.502) AB	8.14 (0.0004)

Table 2. cont.

**BEAVER
POND**

	KIDNEY	LIVER	MUSCLE	F-value (P-value)
METALS				
Al	1.010 (1.953) A	0.129 (2.015) B	8.198 (1.188) C	13.31 (<0.0001)
Ni	0.097 (1.674) A	0.009 (2.146) B	0.132 (1.192) A	6.85 (0.001)
Cu	9.122 (1.147) A	9.573 (1.267) A	2.971 (1.145) B	14.06 (<0.0001)
Se	2.055 (1.466) A	1.407 (1.478) A	0.661 (1.178) A	3.06 (0.053)
Mn	2.564 (1.133) A	3.151 (1.220) A	0.661 (1.198) B	24.51 (<0.0001)
Cd	0.080 (2.082) A	0.032 (1.726) A	0.003 (1.896) B	6.77 (0.002)
Hg	0.071 (1.584) A	0.002 (6.639) A	0.0006 (4.760) A	2.95 (0.058)

**BOGGY
GUT**

	KIDNEY	LIVER	MUSCLE	F-value (P-value)
METALS				
Al	2.381 (1.318) A	0.451 (1.466) B	10.752 (1.087) C	33.49 (<0.0001)
Ni	0.056 (1.436) A	0.008 (1.528) B	0.150 (1.094) A	20.6 (<0.0001)
Cu	10.984 (1.081) A	13.133 (1.058) A	3.662 (1.086) B	88.97 (<0.0001)
Se	3.622 (1.105) A	2.915 (1.086) A	0.422 (1.314) B	45.03 (<0.0001)
Mn	3.056 (1.080) A	5.072 (1.070) B	0.761 (1.085) C	166.92 (<0.0001)
Cd	0.904 (1.218) A	0.182 (1.176) B	0.0003 (1.560) C	193.48 (<0.0001)
Hg	0.114 (1.263) A	0.0005 (3.278) B	0.0002 (3.006) B	12.3 (<0.0001)

Table 3. Percentages of all organs and tissues (kidney, liver, muscle) over the 4 trapping seasons whose concentrations were below detection limits (BDL),in ppm dry weight The Method Detection Limit is in ppm dry weight.

METALS	TB	BP	BG	BDL(ppm)
Al	11.17%	20.83%	12.44%	0.0301
Ni	20.39%	20.83%	19.35%	0.0141
Cu	0.00%	0.00%	0.00%	0.0091
Se	3.88%	2.78%	3.69%	0.0043
Mn	0.00%	0.00%	0.00%	0.0039
Cd	23.79%	17.95%	21.20%	0.0014
U	44.17%	62.50%	92.17%	0.00003
Hg	22.82%	18.06%	20.74%	0.0039

Figure 1.. The Department of Energy's (DOE) Savannah River Site (SRS), established in 1951, is a 777-
km² former nuclear production facility located near Aiken SC, in west-central South Carolina. Location
of experimental and control sites in the north-western part of the site, designated by the circle.

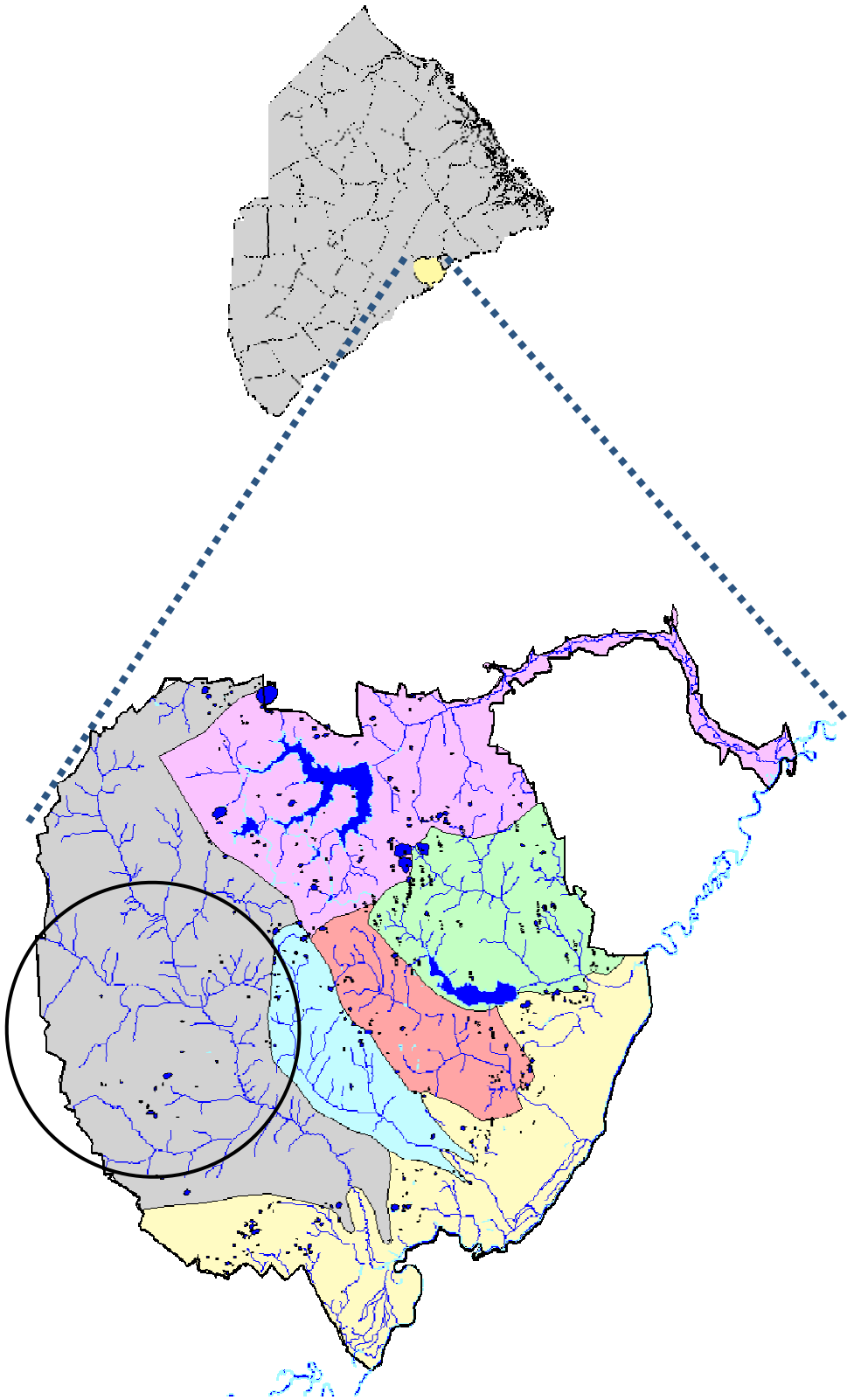


Figure 2. The Steed Pond-Tims Branch riparian corridor (SP-TB) located on the Department of Energy (DOE) Savannah River Site (SRS), located near Aiken, SC USA.

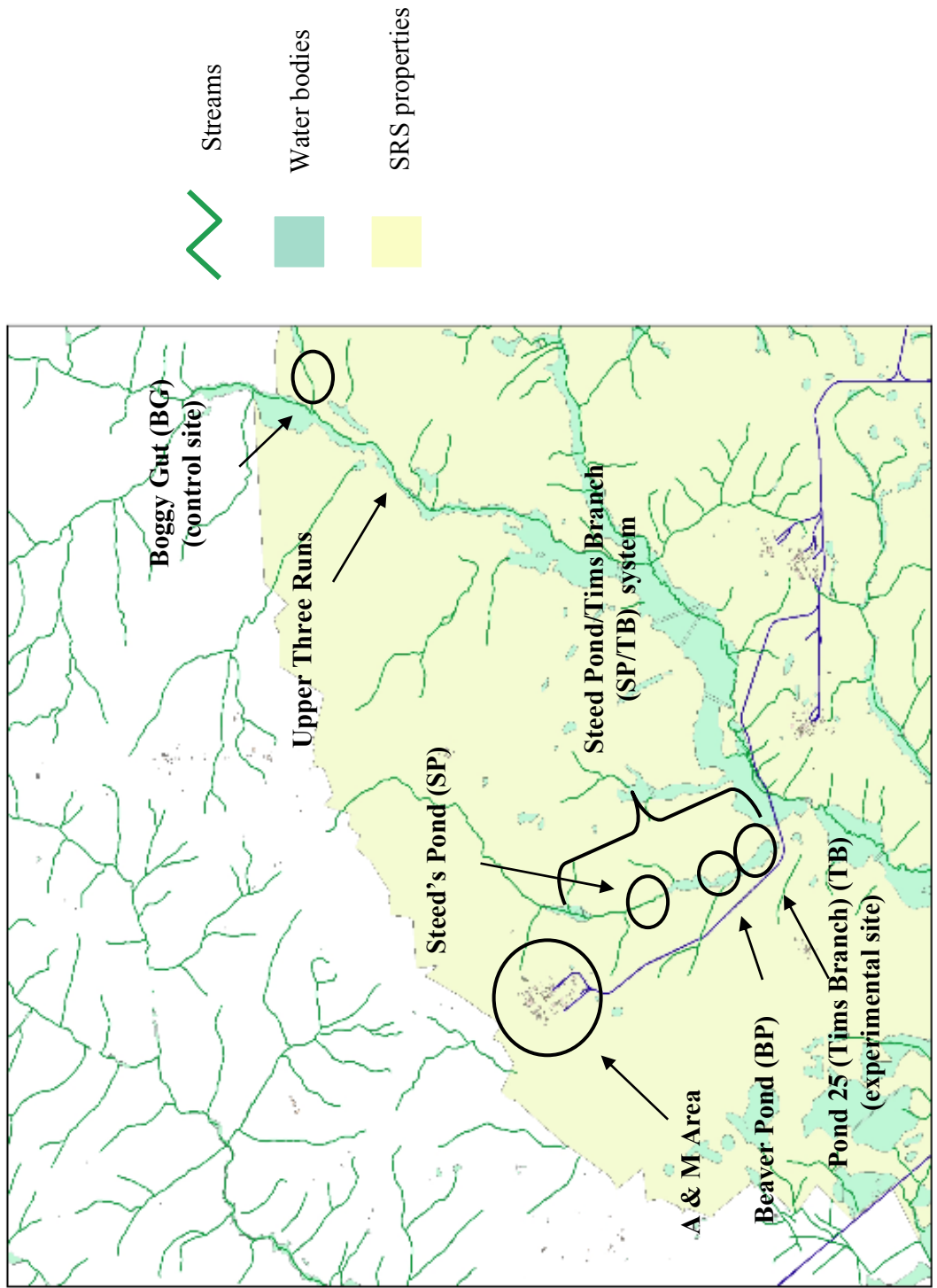


Figure 3. Seasonal differences in metal concentrations (ppm dry weight) in *Peromyscus gossypinus* kidney tissue collected during 2001-2002 on the Savannah River Site Aiken, SC USA. *Asterisk denotes statistical significance at the $p \leq 0.05$ level by ANOVA. **Test could not be performed because of the large number of BDL's (Table 3). The natural logs of the data have been back transformed with the means of concentration represented by the columns with the standard error represented by the bars.

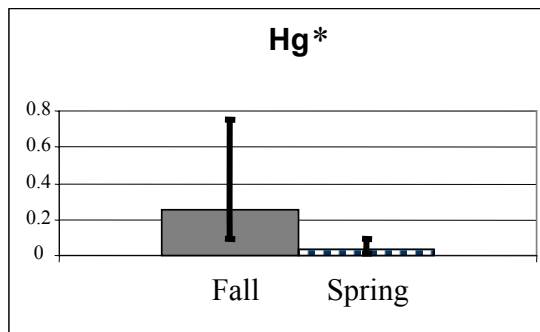
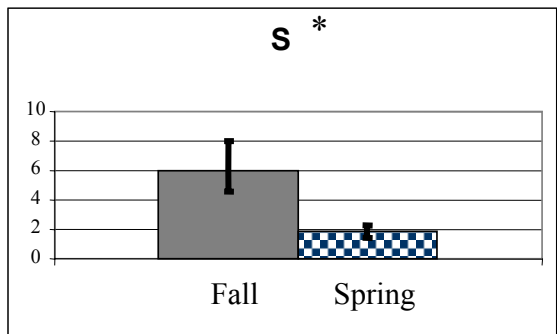
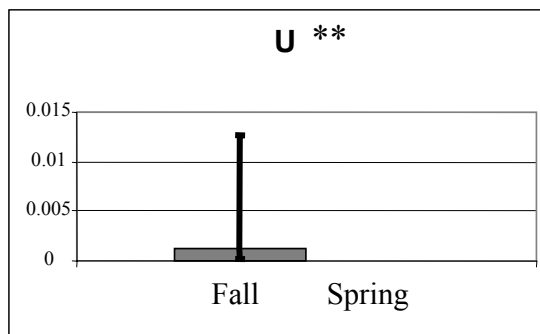
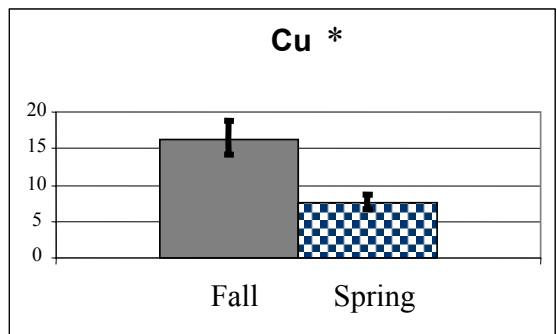
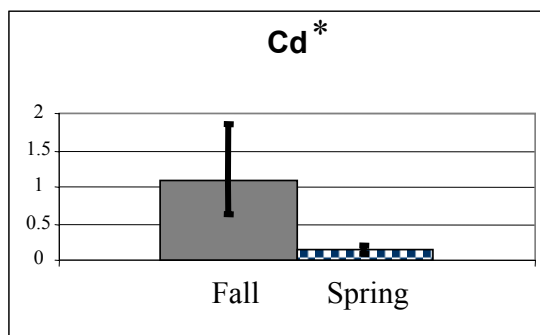
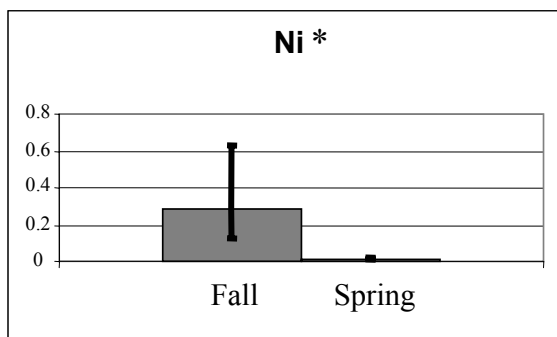
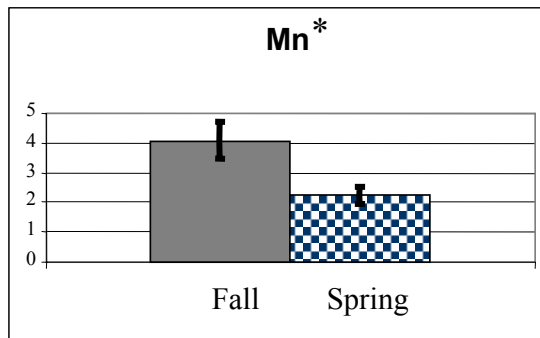
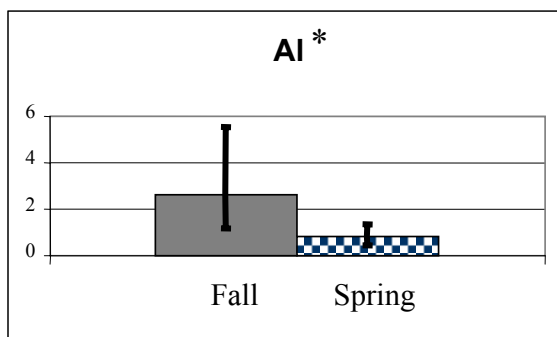


Figure 4. Seasonal differences in metal concentrations (ppm dry weight) in *Peromyscus gossypinus* liver tissue collected during 2001-2002 on the Savannah River Site Aiken, SC USA. *Asterisk denotes statistical significance at the $p \leq 0.05$ level by ANOVA. **Test could not be performed because of the large number of BDL's (Table 3). The natural logs of the data have been back transformed with the means of concentration represented by the columns with the standard error represented by the bars.

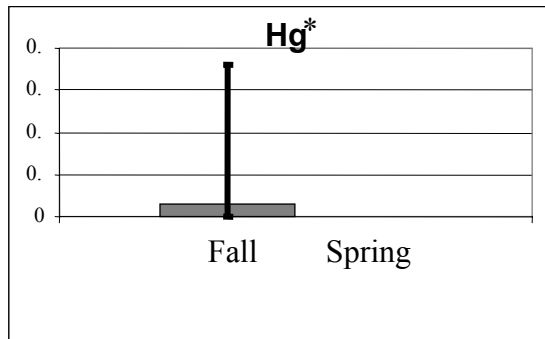
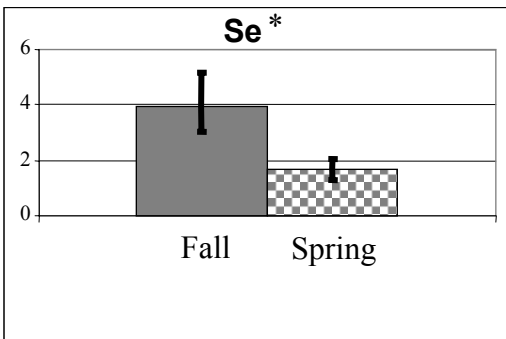
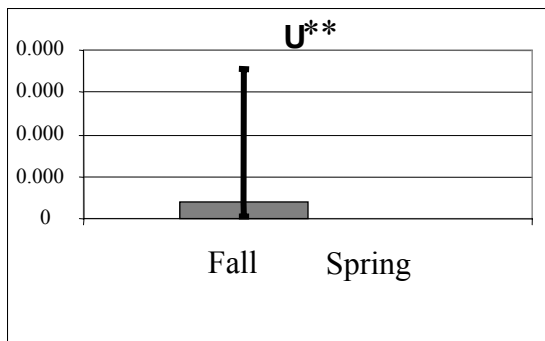
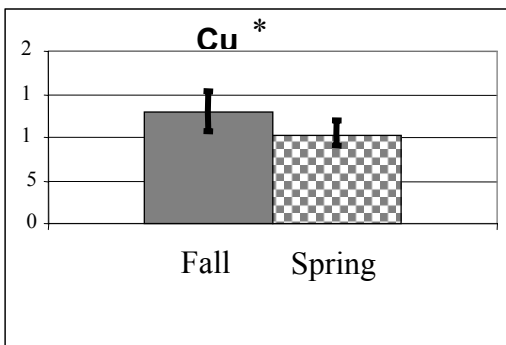
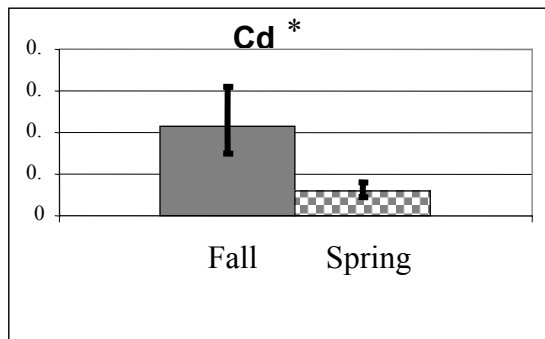
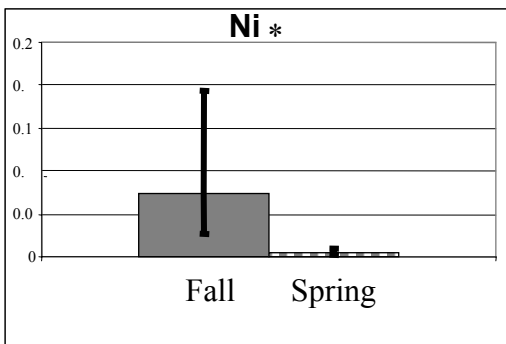
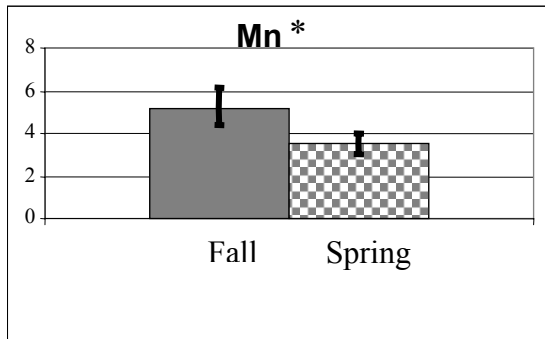
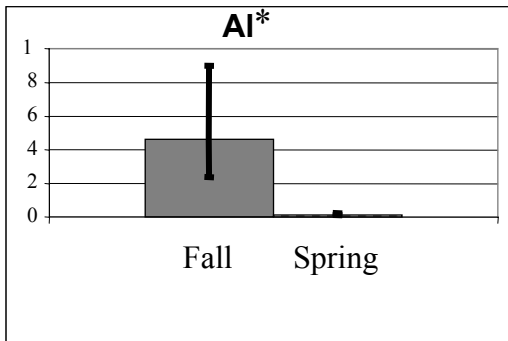


Figure 5. Seasonal differences in metal concentrations (ppm dry weight) in *Peromyscus gossypinus* muscle tissue collected during 2001-2002 on the Savannah River Site Aiken, SC USA. *Asterisk denotes statistical significance at the $p \leq 0.05$ level by ANOVA. **Test could not be performed because of the large number of BDL's (Table 3). The natural logs of the data have been back transformed with the means of concentration represented by the columns with the standard error represented by the bars.

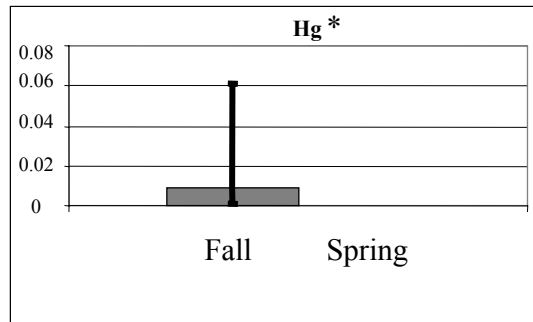
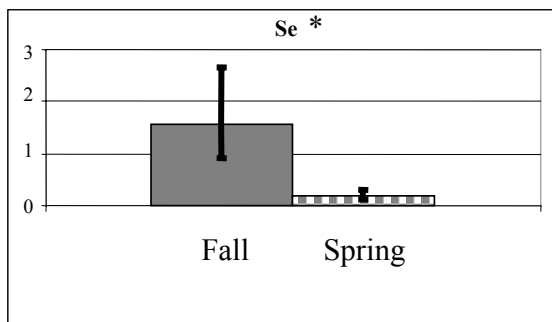
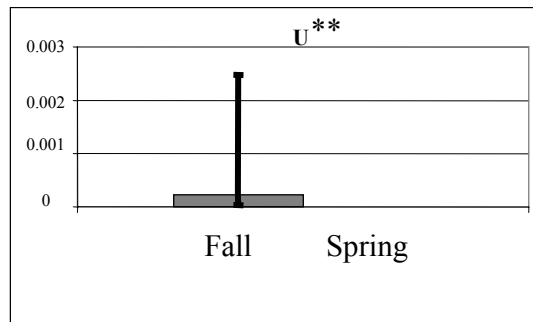
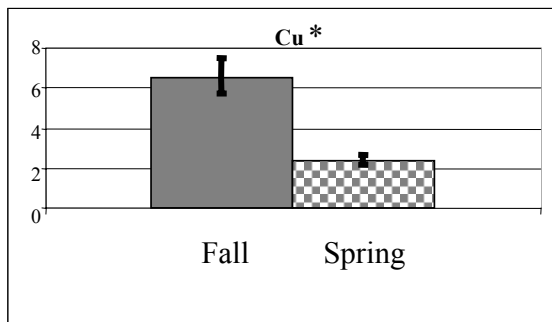
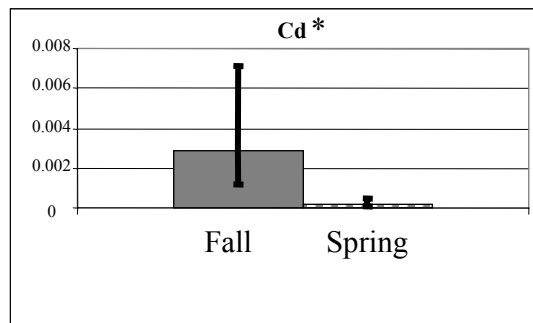
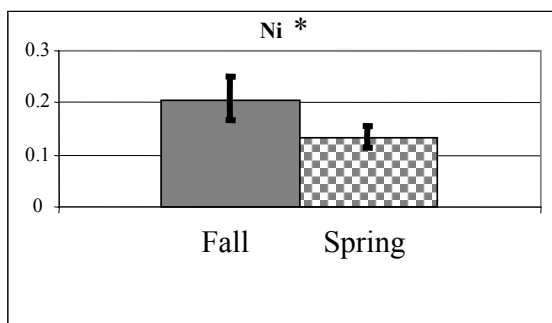
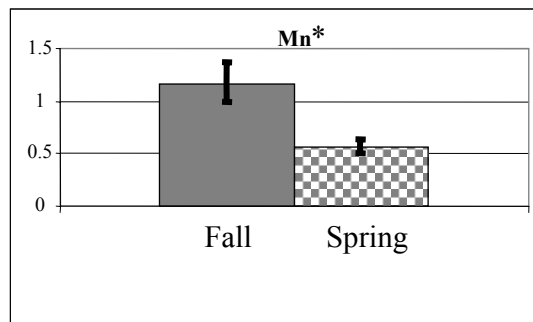
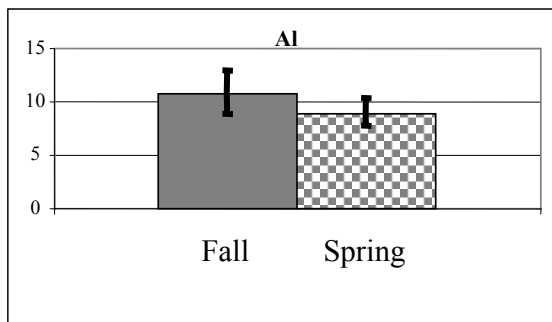


Figure 6. Box plot of $\delta^{15}\text{N}$ levels within species from a small mammal community collected at the SP-TB system located on the Savannah River Site Aiken, SC USA. Species with lower $\delta^{15}\text{N}$ values presumably have diets lower within the food web than those that are higher in $\delta^{15}\text{N}$. Box plots show distribution of data with the horizontal bar located in the box representing the median, the box representing the 25th and 75th percentiles, and 5% and 95% confidence limits expressed as horizontal bars. Outliers are represented by solid circles.

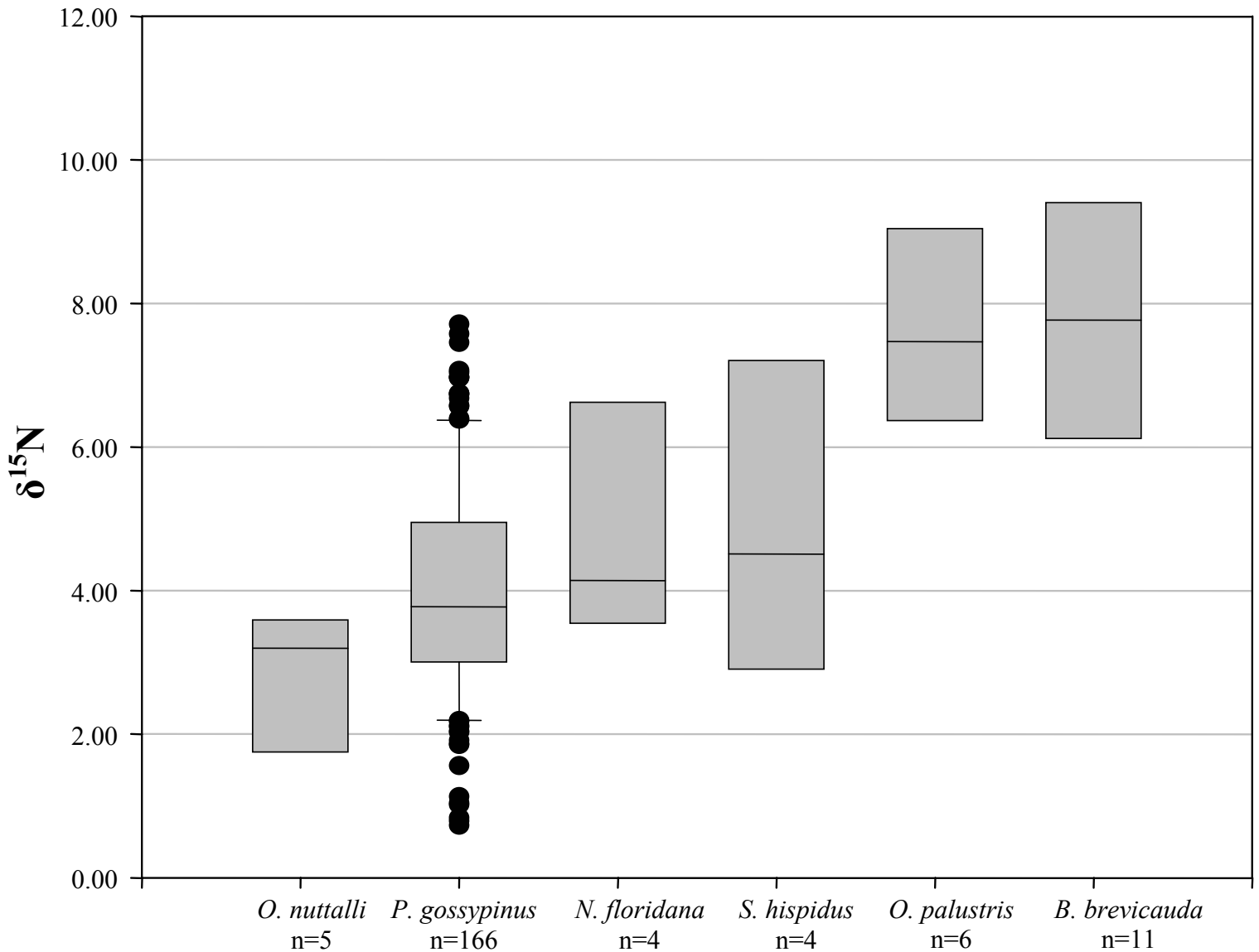


Figure 7. Seasonal variation in $\delta^{15}\text{N}$ values for *P. gossypinus* trapped on the Department of Energy's (DOE) Savannah River Site (SRS) near Aiken, SC USA between the years 2001-2002. Each column represents the mean of $\delta^{15}\text{N}$ values for season with the standard error represented by the bars.

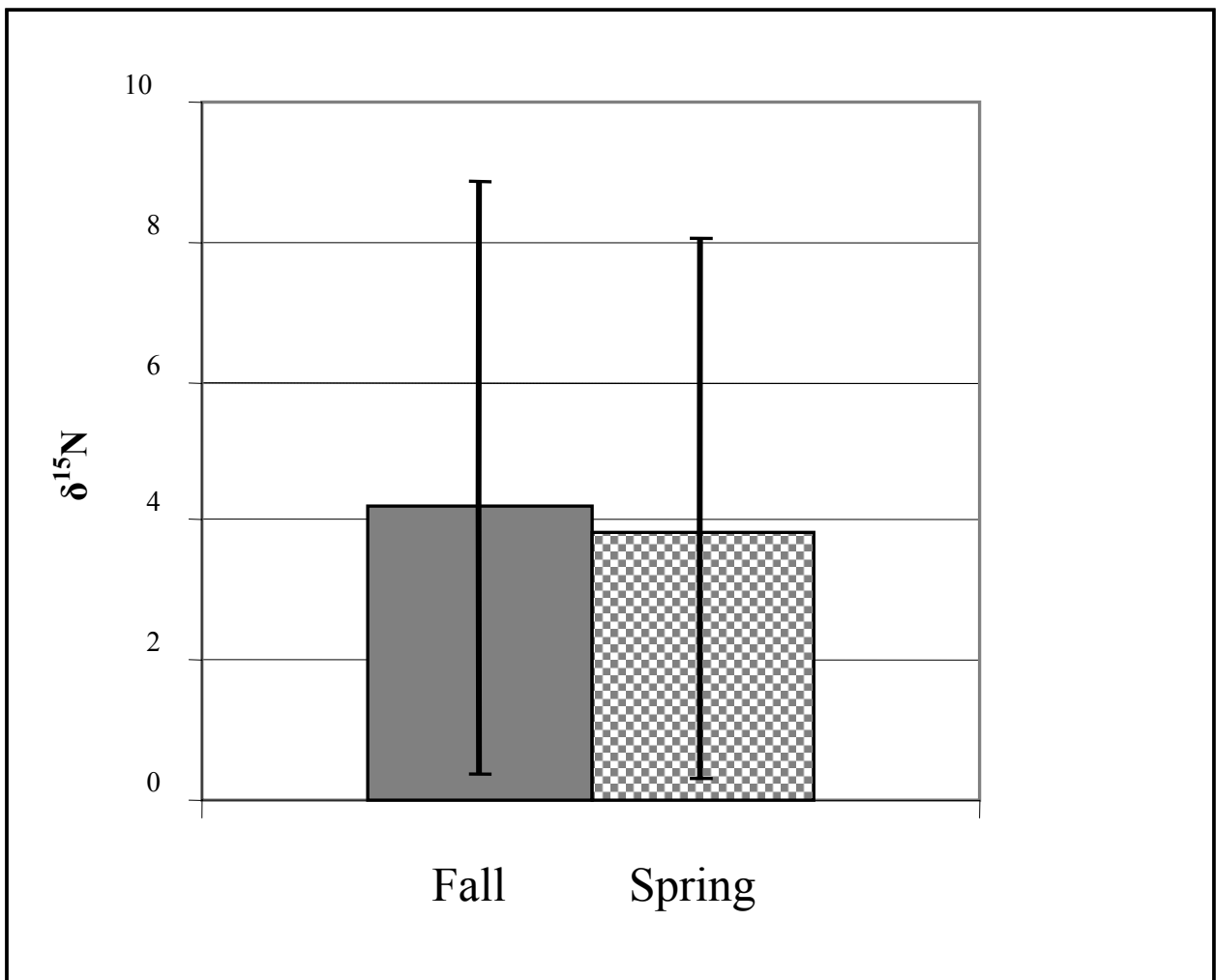


Figure 8. Box plots of $\delta^{13}\text{C}$ from the small mammal community collected at the SP-TB system located on the Savannah River Site Aiken, SC USA. Box plots show distribution of data with the horizontal bar located in the box representing the median, the box representing the 25th and 75th percentiles, and 5% and 95% confidence limits shown as horizontal bars. Outliers are represented by solid circles.

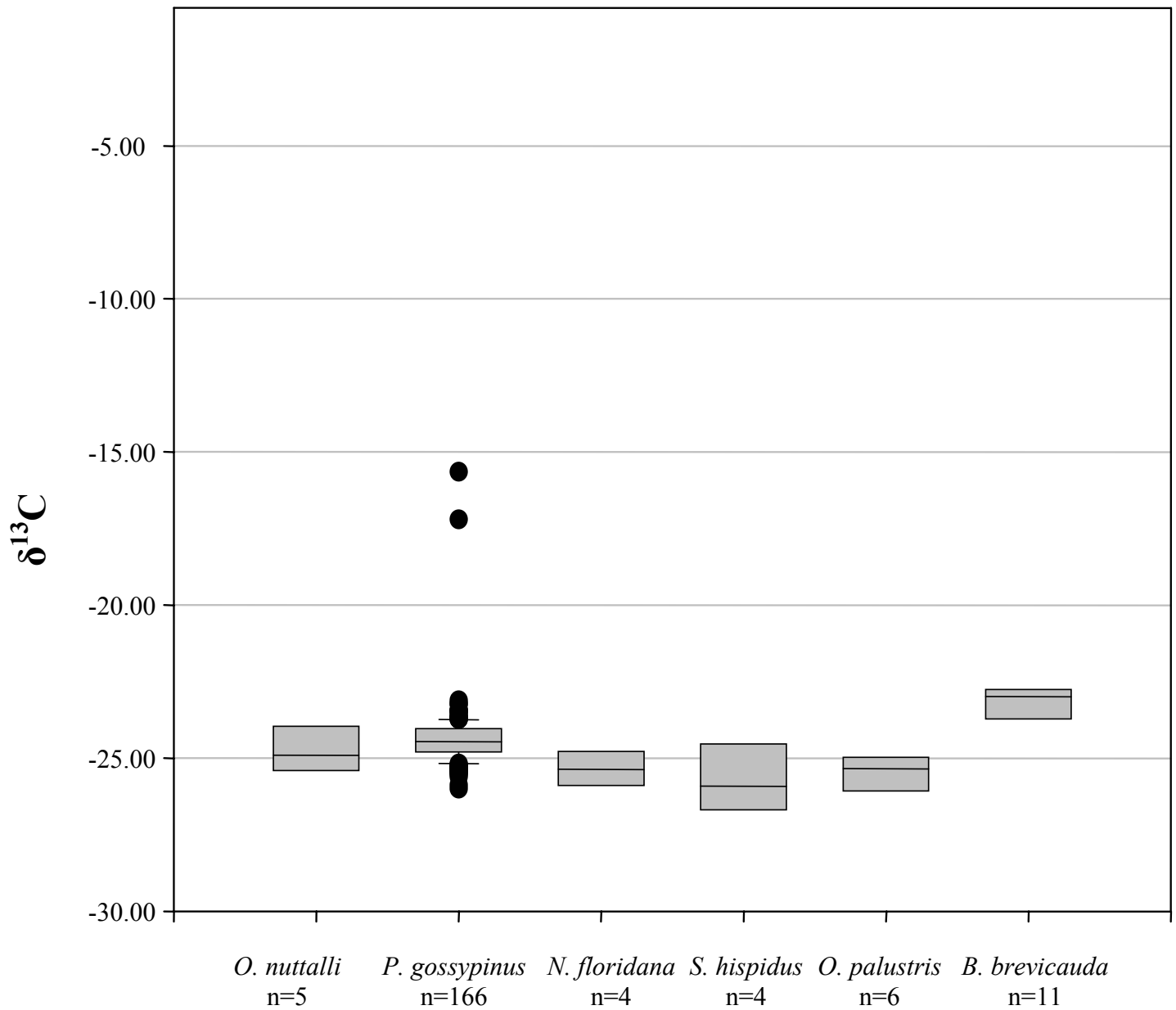
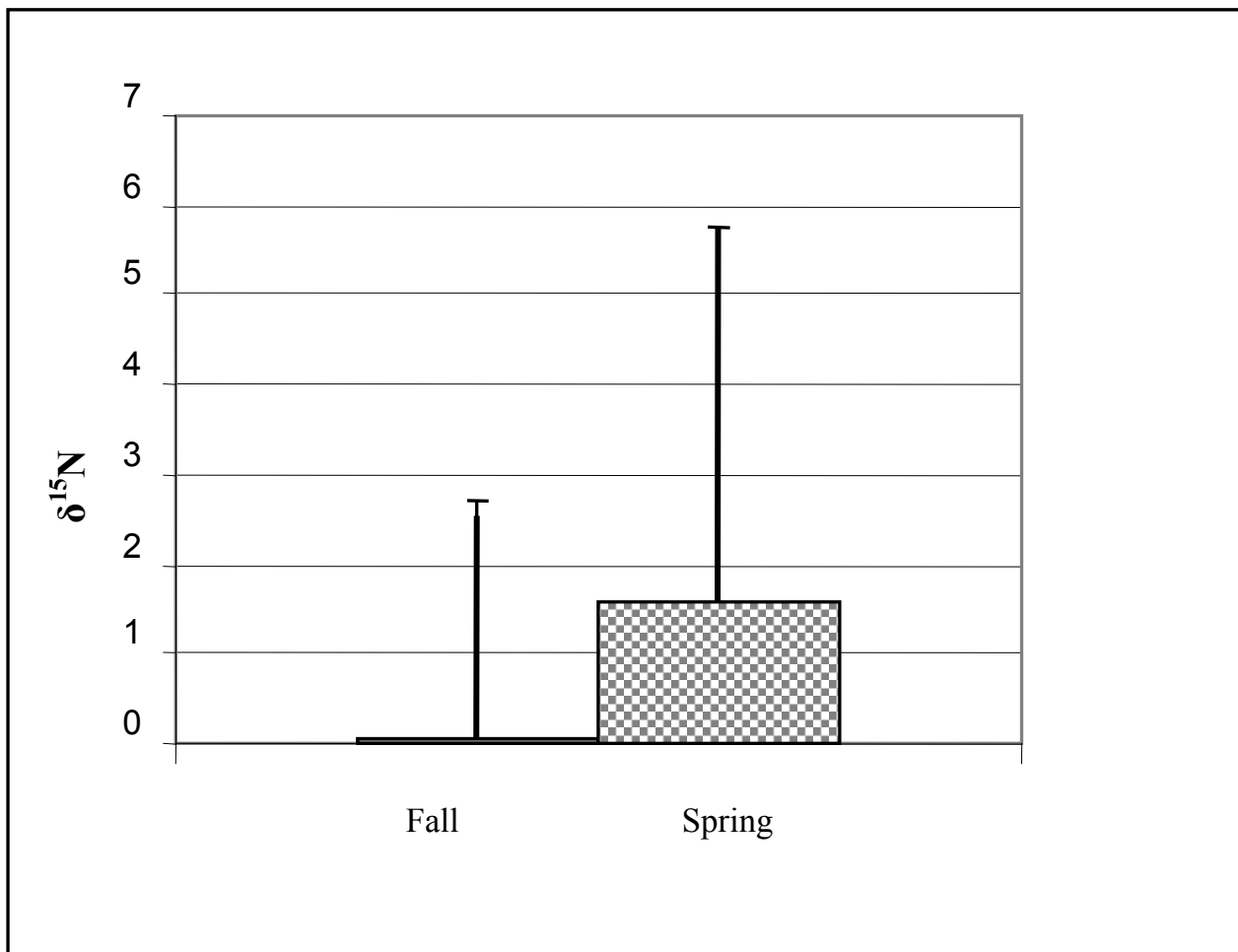


Figure 9. $\delta^{15}\text{N}$ data from possible food items for *Peromyscus gossypinus* collected while trapping was conducted on the Department of Energy's (DOE) Savannah River Site (SRS) near Aiken, SC USA between the years 2001-2002. The average $\delta^{15}\text{N}$ values are represented by the columns with the standard error represented by the bars.



Appendix 1. Summary statistics for metal concentrations in *Peromyscus gossypinus* muscle tissue collected on the Department of Energy's (DOE) Savannah River Site (SRS) located near Aiken, SC USA. All values are Expressed in ppm wet weight.

KIDNEY

Metal	Site	n	Mean	Median	Minimum	Maximum
Al	Tims Branch	69	5.608	2.857	0.000	40.524
	Beaver Pond	24	4.528	4.182	0.000	17.670
	Boggy Gut	73	7.649	4.876	0.004	143.899
Cr	Tims Branch	69	0.531	0.381	0.000	3.882
	Beaver Pond	24	0.406	0.390	0.000	1.820
	Boggy Gut	73	0.353	0.335	0.000	2.253
Co	Tims Branch	69	0.204	0.172	0.055	0.672
	Beaver Pond	24	0.201	0.156	0.121	0.543
	Boggy Gut	73	0.286	0.239	0.045	0.690
Ni	Tims Branch	69	0.199	0.124	0.000	0.899
	Beaver Pond	24	0.373	0.146	0.000	3.241
	Boggy Gut	73	0.273	0.165	0.000	2.015
Cu	Tims Branch	69	13.477	15.972	3.981	22.485
	Beaver Pond	24	11.200	12.711	3.786	29.950
	Boggy Gut	73	13.040	16.053	3.694	23.801
Se	Tims Branch	69	4.430	5.213	0.001	8.410
	Beaver Pond	24	3.881	4.456	0.001	10.494
	Boggy Gut	73	4.761	5.253	0.566	13.919
Mn	Tims Branch	69	3.601	3.994	0.952	6.336
	Beaver Pond	24	3.039	3.446	1.119	7.099
	Boggy Gut	73	3.648	3.932	1.002	8.268
Cd	Tims Branch	69	1.214	0.817	0.000	8.446
	Beaver Pond	24	0.538	0.326	0.000	2.048
	Boggy Gut	73	1.906	1.155	0.000	8.464
Pb	Tims Branch	69	0.538	0.342	0.055	2.109
	Beaver Pond	24	0.453	0.352	0.091	1.038
	Boggy Gut	73	0.692	0.345	0.000	5.164
U	Tims Branch	69	0.065	0.031	0.000	0.718
	Beaver Pond	24	0.026	0.000	0.000	0.126
	Boggy Gut	73	0.000	0.000	0.000	0.015
Hg	Tims Branch	69	0.281	0.229	0.000	0.977
	Beaver Pond	24	0.174	0.177	0.000	0.490
	Boggy Gut	73	0.223	0.206	0.000	0.955

Appendix 1 cont.

LIVER

Metal	Site	n	Mean	Median	Minimum	Maximum
Al	Tims Branch	69	5.833	2.437	0.000	112.909
	Beaver Pond	24	2.147	0.734	0.004	11.541
	Boggy Gut	73	3.693	1.487	0.004	26.480
Cr	Tims Branch	69	0.568	0.225	0.000	24.242
	Beaver Pond	24	0.129	0.114	0.000	0.407
	Boggy Gut	73	0.361	0.131	0.000	10.784
Co	Tims Branch	69	0.133	0.123	0.071	0.328
	Beaver Pond	24	0.154	0.152	0.000	0.425
	Boggy Gut	73	0.223	0.191	0.062	0.677
Ni	Tims Branch	69	0.169	0.145	0.000	0.934
	Beaver Pond	24	0.189	0.088	0.000	0.539
	Boggy Gut	73	0.160	0.087	0.000	2.159
Cu	Tims Branch	69	12.899	14.515	3.798	26.714
	Beaver Pond	24	12.059	11.540	0.049	26.104
	Boggy Gut	73	14.789	13.495	4.797	49.414
Se	Tims Branch	69	2.660	2.5730	0.671	6.120
	Beaver Pond	24	2.579	2.189	0.000	6.411
	Boggy Gut	73	3.653	3.884	0.853	12.370
Mn	Tims Branch	69	5.730	5.461	1.373	38.182
	Beaver Pond	24	4.104	3.378	0.067	11.303
	Boggy Gut	73	5.958	5.394	1.673	15.010
Cd	Tims Branch	69	0.252	0.164	0.026	2.412
	Beaver Pond	24	0.106	0.051	0.000	0.329
	Boggy Gut	73	0.325	0.225	0.000	2.108
Pb	Tims Branch	69	0.205	0.098	0.000	3.483
	Beaver Pond	24	0.146	0.130	0.027	0.335
	Boggy Gut	73	0.181	0.116	0.000	1.121
U	Tims Branch	69	0.009	0.000	0.000	0.221
	Beaver Pond	24	0.001	0.000	0.000	0.014
	Boggy Gut	73	0.004	0.000	0.000	0.296
Hg	Tims Branch	69	0.870	0.051	0.000	53.723
	Beaver Pond	24	0.085	0.074	0.000	0.237
	Boggy Gut	73	0.065	0.043	0.000	0.323

Appendix 1 cont.

MUSCLE

Metal	Site	n	Mean	Median	Minimum	Maximum
Al	Tims Branch	69	11.497	10.149	2.101	32.650
	Beaver Pond	24	11.040	8.824	1.064	33.137
	Boggy Gut	73	13.528	9.625	1.780	56.426
Cr	Tims Branch	69	0.444	0.361	0.000	1.482
	Beaver Pond	24	0.389	0.251	0.000	1.213
	Boggy Gut	73	0.678	0.415	0.000	12.279
Co	Tims Branch	69	0.035	0.029	0.015	0.119
	Beaver Pond	24	0.548	0.056	0.021	1.897
	Boggy Gut	73	0.074	0.036	0.016	1.562
Ni	Tims Branch	69	0.253	0.175	0.042	0.962
	Beaver Pond	24	0.175	0.149	0.020	0.379
	Boggy Gut	73	0.231	0.144	0.042	1.500
Cu	Tims Branch	69	4.504	5.095	1.328	7.945
	Beaver Pond	24	3.690	1.919	1.382	8.544
	Boggy Gut	73	4.550	5.033	1.288	13.018
Se	Tims Branch	69	0.887	0.956	0.001	1.867
	Beaver Pond	24	0.873	1.051	0.205	2.146
	Boggy Gut	73	1.003	1.082	0.001	2.493
Mn	Tims Branch	69	0.968	0.980	0.218	2.737
	Beaver Pond	24	0.961	0.686	0.129	4.803
	Boggy Gut	73	0.980	0.861	0.157	4.746
Cd	Tims Branch	69	0.026	0.000	0.000	0.240
	Beaver Pond	24	0.061	0.005	0.000	0.353
	Boggy Gut	73	0.032	0.000	0.000	0.313
Pb	Tims Branch	69	0.106	0.082	0.000	0.579
	Beaver Pond	24	0.156	0.109	0.028	0.668
	Boggy Gut	73	0.116	0.070	0.000	0.508
U	Tims Branch	69	0.027	0.009	0.000	0.300
	Beaver Pond	24	0.009	0.005	0.000	0.066
	Boggy Gut	73	0.001	0.000	0.000	0.012
Hg	Tims Branch	69	0.048	0.046	0.000	0.193
	Beaver Pond	24	0.030	0.017	0.000	0.106
	Boggy Gut	73	0.044	0.027	0.000	0.192