ANTHROPOGENIC CONTAMINATION IN SEMI-AQUATIC MAMMALS IN THE SOUTHEASTERN UNITED STATES: IMPACT OF CONTAMINANT BURDENS ON PARASITE COMMUNITIES AND INFLUENCE OF SPACE USE ON CONTAMINANT UPTAKE

by

ERNEST J. BORCHERT

(Under the Direction of James C. Beasley)

ABSTRACT

Environmental contamination by anthropogenic pollutants is a common issue worldwide. Given their association with aquatic systems, semi-aquatic mammals are useful for monitoring contaminants and may be important indicators of ecosystem health. The objectives of this research are to determine trace element and radionuclide levels in tissues of raccoons, beaver, and otter collected in the southeastern United States, and elucidate the influence of contaminant exposure on endoparasite communities in these species. Additionally, we use raccoon GPS data to model how fine-scale habitat use influences exposure to a heterogeneously distributed contaminant. The results of this research revealed interspecific-differences in contaminant exposure and that spatial variability in contaminant distribution exists in the southeastern U.S. Further, animal use of contaminated environments was positively associated with their measured contaminant burdens. Collectively, this research suggests semi-aquatic mammals are useful bioindicators, but spatial heterogeneity in use of contaminated environments exists among individuals, even at highly localized scales.

INDEX WORDS: Biomagnification, Furbearer, Habitat Selection, Home Range, Mercury, Radiocesium

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B.S., State University of New York College of Environmental Science and Forestry, 2013

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2018

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May 2018

DEDICATION

This is dedicated to my mother Patricia and father Ernest. Without your support none of this would be possible.

ACKNOWLEDGEMENTS

There are so many people who helped me along the way and without them none of this would be possible. First, I want to thank my advisor Jim Beasley for giving me a chance and his guidance throughout this process. When I came to work for you as a technician three years ago, I had no idea I would become one of your students, thank you. I want to thank Larry Bryan for this constant advice and support and for showing me the ecotox-ropes. You were always looking out for me. I would like to thank my committee members Guha Dharmarajan and Michael Yablsey who provided invaluable expertise. Also, a big thank you needs to go to my fellow Beasley Lab and SREL members who have made this feel like a home away from home. I would like to thank Kevin Eckert who checked raccoon traps for me when I couldn’t. You didn’t catch anything, but you tried! Also thanks to Zak Smith, who caught me a bunch of otters from SC. I would like to thank USDA-APHIS for all their help in sample collection. Last but not least, I would like to thank Chris Leaphart who helped me every step of the way.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The degradation of Earth's ecosystems due to anthropogenic disturbance occurs worldwide due to activities such as intensive grazing, habitat fragmentation, overfishing, the introduction of invasive species, and altering biogeochemistry (Vitousek et a. 1997). Humans impact Earth's biogeochemistry in several ways including alterations to the carbon cycle, water cycle, nitrogen cycle, and the mobilization of metals that exceeds their natural fluxes (Vitousek et al. 1997). This includes the mobilization of environmental contaminants, such as trace elements and radionuclides, a common issue throughout the world that threatens ecosystem structure and function (Rhind 2009, Coolon et al. 2010, Johnston et al. 2015).

Ecosystem inputs of trace elements originate from both natural and anthropogenic sources. Natural sources include the weathering of soils, wildfires, and geothermal activity, whereas typical anthropogenic sources include metal mining, coal combustion, industrial operations and waste disposal (Swaine 2000). Once trace elements enter the environment they can bioaccumulate in the tissues of organisms. Thus, trace elements such as mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), cesium (Cs), copper (Cu), nickel (Ni), lead (Pb), selenium (Se), uranium (U), aluminum (Al), and zinc (Zn) and radionuclides such as ^{137}Cs pose a potential threat to both human and wildlife health (Domingo 1994, French et al. 1974, Wolfe et al. 1998, Scheuhammer et al. 2007). Concentration levels are determined by the rate of uptake minus the rate of elimination, including biotransformation and excretion (Barron 1995; Reinfelder et al. 1998). In addition to bioaccumulation, certain trace elements such as Hg, Rb, and in some cases Se are known to biomagnify within food webs, resulting in higher concentrations with increased trophic position (Brown and Luoma 1995, Sydeman and Jarman 1998, Campbell et al. 2005, Ikemoto et al. 2008, Nfon et al. 2009).

^{137}Cs is a radioactive isotope of cesium (Cs) with a physical half-life of 30.2 years (Paller et al. 2014). In addition to a physical half-life, radio-contaminants also exhibit an ecological half-life, which is a measure of persistence in an ecosystem, and a biological half-life, which is a measure of persistence within an organism after exposure is discontinued. Both ecological half-life (time it takes for 50% to become unavailable in an ecosystem) and biological half-life (time it takes for 50% of a substance to be eliminated through metabolic turnover or excretion) vary based on emergent properties within ecosystems and the species in question, and may differ from the actual physical half-life of an element (Paller et al. 2014, Fendley et al. 1977). For example, Paller et al. (2014) found that the ecological half-life of ^{137}Cs on the Savannah River Site was 15.9 years for white-tailed deer (*Odocoileus virginianus*) and 3.5-9.0 years for fish, far below the actual physical half-life for this element. Additionally, previous studies looking at the biological half-life of ^{137}Cs in various wildlife species found a biological half-life of 5.6 days for Wood Ducks (*Aix sponsa*) and 30.1 days for green tree frogs (*Hyla cinerea*), suggesting rapid elimination occurs within body tissues upon cessation of exposure (Dapson and Kaplan 1975, Fendley et al. 1977).

As a naturally occurring element, ^{137}Cs is found in small quantities within the environment, but is most well known as a product of nuclear fission. Massive acute inputs of ^{137}Cs have occurred due to nuclear accidents at Chernobyl and the Fukushima

Daiichi Nuclear Power Plant, as well as through nuclear weapons testing. For example, the Chernobyl accident in 1986 released approximately 5200 PBq of total radiation, of which 47 PBq was ^{137}Cs (Steinhauser et al. 2014); the Fukushima Daiichi accident in 2011 released approximately 520 PBq of total radiation, of which 11.8 PBq was ^{137}Cs (Steinhauser et al. 2014). Other inputs include low doses produced by nuclear industrial activities (Jannik 1999).

Trace elements are important when considering wildlife and ecosystem health due to their deleterious effects at high concentrations. Effects on individuals include organ damage, disruption of the nervous system, reproductive/fertility problems, decreased immunocompetence, and death (Govind & Madhuri 2014). At the population level, these effects can manifest as decreased fecundity and survivorship, resulting in population declines.

Semi-aquatic mammals such as the North American river otter (*Lontra canadensis*), North American beaver (*Castor canadensis*), and raccoon (*Procyon lotor*) make good species for ecotoxicology studies for several reasons. First, they move freely between contaminated aquatic systems and terrestrial systems, representing a potential vector for contaminate transfer between the two. Second, these species represent multiple trophic levels at which toxicological effects can be determined (Klenavic et al. 2008, Lotze and Anderson 1979, Novak 1987). Third, beavers are considered a keystone species and have great effects on ecosystems (Mills et al. 1993).

One aspect of ecotoxicology and environmental health where there are substantive data gaps, is how elevated trace element and radionuclide levels in mammalian hosts influence parasite communities. Several studies have suggested parasite communities are sensitive to ecological disturbances and could be important indicators of ecosystem health (Poulin 1992, Dusek et al. 1998, MacKenzie 1999, Huspeni and Lafferty 2004, Marcogliese 2005). Parasites are useful indicators of ecosystem health because there are more parasites than free living organisms on Earth, they show incredible biodiversity due to adaptations to different hosts and environments (MacKenzie 1999), parasites have complex life cycles with different biological requirements, and most parasites have a short free-living stage which makes them particularly sensitive to ecological disturbances (MacKenzie 1999).

Most studies assessing the utility of parasites as indicators of ecosystem health have occurred in aquatic systems (Poulin 1992, Dusek et al. 1998, MacKenzie 1999, Huspeni and Lafferty 2004, Marcogliese 2005). For example, salt mash restoration has been shown to increase the prevalence and diversity of digeneans parasitizing the California horn snail (*Cerithidea californica*) (Huspeni and Lafferty 2004). Huspeni and Lafferty (2004) attributed the increased digenean prevalence and diversity parasitizing California horn snails to changes in bird use at the restored habitat, because birds are a potential definitive host for many trematode species (Huspeni and Lafferty 2004). Additionally, when examining parasite diversity of chubs (*Leuciscus cephalus*) in freshwater lakes, assemblages of specialist parasites in polluted lakes exhibited significantly reduced species richness, whereas species richness of generalist parasites increased (Dusek et al. 1998). To further complicate matters, it is possible parasite infection could potentially increase in polluted systems due to host immunosuppression (Poulin 1992). To date, there have been few published studies that address how trace elements affect parasite communities in terrestrial systems (Klenavic et al. 2008, McGrew et al. 2015, Binkowski et al. 2016), only one of which looked at how trace element contamination effects parasite diversity (Hernandez et al. 2017).

An additional area of interest in ecotoxicology is how an animal's space use and resource selection patterns influence contaminant uptake. Both the concentration and distribution of environmental contaminants can vary extensively throughout the landscape, even at fine spatial scales (Taylor et al. 2005, Purucker et al. 2007). This spatial heterogeneity of contaminants causes both inter- and intra-specific differences in contaminant exposure. One way to gain a better understanding of how the habitat use of wildlife affect their contaminant concentrations is through simultaneous collection of movement and contaminant exposure data. Several recent studies have incorporated telemetry data to gain a better understanding of contaminant exposure and uptake at large spatial scales (Ackerman et al. 2008, Yates et al. 2010, Fort et al. 2014, Peterson et al. 2015, Hinton et al. 2015). For example, telemetry data has been used to describe how the space use of pre-breeding American Avocets (*Recurvirostra americana*) and Black-necked Stilts (*Himantopus mexicanus*) influenced mercury concentrations (Ackerman et al. 2008). Their study found that variation in blood mercury concentrations between sites was due to differences in foraging areas, habitat use, and foraging strategies (Ackerman et al. 2008).

Additionally, telemetry has been useful in explaining variation in mercury concentrations in little auks (*Alle alle*) due to differences in habitat use during the non-breeding season (Fort et al. 2014). Previous studies have used telemetry data to assess how foraging ecology in northern elephant seals (*Mirounga angustirostris*) influences mercury bioaccumulation, and helped identify exposure of White-faced Ibis (*Plegadis* *chihi*) to persistent organic pollutants at wintering areas (Yates et al. 2010, Peterson et al. 2015). Recently, GPS telemetry has been paired with dosimetry technology to allow researchers to directly measure radiological exposure in relation to animal movements (Hinton et al. 2015). With the growing popularity of satellite telemetry, it is becoming possible to examine how space use at fine spatio-temporal scales influences contaminant exposure. This is of particular importance when contaminants vary at small spatial scales are the subject of interest.

The goal of my thesis research was to gain a better understanding of wildlife exposure to a broad suite of contaminants in the southeastern U.S., and how animal space use influences contaminant uptake. This was accomplished by answering two disparate research questions. First, in Chapter 2 I determined how the past and present industrial operations of the Savannah River Site are affecting contaminant concentrations in river otter (*Lontra canadensis*), beaver (*Castor canadensis*), and raccoons (*Procyon lotor*), how these levels compare to animals collected across South Carolina and Georgia, and how contaminant concentrations vary relative to trophic position. I also investigated the correlation between trace element and radionuclide concentrations in semi-aquatic mammalian hosts and their endoparasite communities. This is one of the first studies to look at this relationship across a large spatial extent. The reason for using otter, beaver, and raccoons as model species is because they can move freely between contaminated aquatic systems and terrestrial systems, transporting contaminants between the two, and represent multiple trophic levels at which toxicological effects can be assessed (Klenavic et al. 2008, Lotze and Anderson 1979, Novak 1987). Additionally, there is a general lack of recent contaminant data for furbearers in the Southeast United States as most studies are over 25 years old (Jenkins et al. 1969, Cumbie and Jenkins 1974, Cumbie 1975a, Cumbie 1975b, Carmichael and Baker 1989). Second, in Chapter 3 I use satellite telemetry to determine how fine scale habitat use of animals is related to contaminant uptake, using raccoons as a model organism and ^{137}Cs as a model contaminant. This information will be useful not only in regards to areas contaminated with ^{137}Cs, but applies to any form of environmental contamination that exhibits heterogeneous distribution at fine spatial scales.

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CHAPTER 2

TRACE ELEMENTS AND RADIOCESIUM IN SEMI-AQUATIC MAMMALS IN THE SOUTHEASTERN UNITED STATES AND THEIR IMPACT ON HOST ENDOPARASITE COMMUNITIES

Borchert, E.J., A.L. Bryan Jr., J.C. Leaphart, and J.C. Beasley. To be submitted to Science of the Total Environment.

ABSTRACT

Environmental contamination of trace elements, radionuclides, and other contaminants is a pervasive issue throughout the world. Many contaminants can persist in the environment for decades or more after their initial deposition, and can biomagnify in organisms based on their trophic position. This study seeks to quantify radionuclide (^{137}Cs) and trace element (Hg, Cd, Cu, Fe, K, Mg, Ni, Pb, Zn, Cr, As, Se, U, Mn, Sr) concentrations in three semiaquatic mammals of different trophic position: North American river otter (*Lontra canadensis*), raccoon (*Procyon lotor*), and North American beaver (*Castor Canadensis*). These mammals were collected throughout a portion of the southeastern United States, and used to determine how host trace element and radionuclide concentrations influence endoparasite communities. Muscle and liver samples were taken from animals collected from the U.S. Department of Energy's Savannah River Site (SRS) (South Carolina, USA) and other locations throughout South Carolina and Georgia. Mercury was the only element to clearly demonstrate biomagnification based on trophic position, although we observed differences in concentrations of other trace elements and ^{137}Cs among species and sampling sites. Mercury concentrations in otters were generally higher than previous studies conducted in the northern U.S. and Canada, but our data revealed differences among our sampling sites, with samples collected from the Coastal Plain having higher concentrations than samples collected from the Piedmont region. Additionally, our results suggest exposure to Hg may play a potential role in endoparasite abundance and Se exposure may influence endoparasite diversity. These findings further demonstrate how wildlife can

accumulate anthropogenic contamination, and how this contamination can influence ecological processes.

INTRODUCTION

Humans have profound impacts on Earth's ecosystems at a variety of spatiotemporal scales, ranging from acute localized effects to chronic global impacts. Through mechanized agriculture, introduction of invasive species, deforestation, overexploitation, and pollution, humans have dramatically altered ecosystem structure and function (Vitousek et al. 1997). This includes the mobilization of trace elements and radionuclides in ways that exceed their natural fluxes, posing a potential threat to both human and wildlife health (French et al. 1974, Wolfe et al. 1998, Scheuhammer et al. 2007). In particular, anthropogenic pollution is widespread throughout the world, and understanding its effects is crucial to ensuring environmental integrity (Rhind 2009).

Trace elements originate from both natural and anthropogenic sources including atmospheric deposition, geothermal activity, mining, coal combustion, and industrial operations (Swaine 2000). Once trace elements enter the environment they can bioaccumulate within various tissues of organisms, and in some cases biomagnify within food webs (Brown and Luoma 1995, Sydeman and Jarman 1998, Campbell et al. 2005, Ikemoto et al. 2008, Nfon et al. 2009). Similarly, radionuclides are a class of environmental contaminants that release radiation as they undergo radioactive decay and accumulate within plant and animal tissues. Although radionuclides are naturally found in the environment, substantive anthropogenic inputs of ^{137}Cs, a product of nuclear fission, have been introduced globally through nuclear weapons testing and nuclear accidents at the Chernobyl and Fukushima Daiichi nuclear power plants, which released

47 PBq of ^{137}Cs (5200 PBq of total radiation) and 11.8 PBq of ^{137}Cs (5200 PBq of total radiation), respectively (Steinhauser et al. 2014). Additional radionuclides have been introduced through normal operations of nuclear power plants, although these inputs represent a small portion of all radionuclides released into the environment through anthropogenic activities (Jannik 1999).

Once introduced into a system, anthropogenic contaminants can impact the health, physiology, and ultimately survival of biota (Scheuhammer et al. 2007). Trace elements (Hg, Cr, Ni, Cu, Zn, As, Se, Cd, Pb, U) are of particular concern when considering wildlife health due to their potential effects at high concentrations, including weight loss, ataxia, lethargy, decreased reproductive success, paralysis, and even death (Scheuhammer et al. 2007, Degernes 2008). The effects of radionuclides, however, are less clear (Beresford and Copplestone 2011, Bréchignac et al. 2016). For example, while some studies have reported negative effects in some organisms inhabiting the contaminated landscape surrounding Chernobyl (Moller et al. 2005), other studies suggest robust mammal communities now exist in Chernobyl (Baker et al. 1996, Deryabina et al. 2015, Webster et al. 2016), although the health of these organisms remains understudied.

Bioindicators have long been used to detect the effects of anthropogenic pollution and evaluate ecosystem health. However, many taxa used as bioindicators are ecosystem-specific. Parasites are potentially important indicators of ecosystem health as they are highly diverse, globally distributed, and parasite communities are sensitive to ecological disturbances (Poulin 1992, Dusek et al. 1998, MacKenzie 1999, Huspeni and Lafferty 2004, Marcogliese 2005, Hudson et al. 2006, Sures et al. 2017). Indeed, parasites accumulate various types of environmental contaminants at higher concentrations than their hosts, including trace elements and persistent organic pollutants (Galli et al. 1998, McGrew et al. 2015, Oluoch-Otiego et al. 2016, Yen Le et al. 2014). Parasite community structure also has been shown to change in response to anthropogenic pollution, due to direct toxic effects and the absence of intermediate hosts in degraded systems (Lafferty 1997, Overstreet 1997, MacKenzie 1999, Huspeni and Lafferty 2004, Marcogliese 2004). Parasite communities in polluted systems shift from heteroxenous (parasites requiring at least two hosts) and specialist parasites to monoxenous (parasites requiring a single host) and generalist parasites (Dusek et al. 1998, Perez-del Olmo et al. 2007, Sures et al. 2017). However, there is some thought total parasite burdens may increase due to pollutants compromising host immunity (Poulin 1992, Overstreet 1997, MacKenzie 1999, Marcogliese 2004). Additionally, parasite diversity has been shown to be inversely related to ecological disturbance (Chapman et al. 2015, Huspeni and Lafferty 2004).

While there are many studies exemplifying the use of parasites as bioindicators, most have occurred in aquatic systems using fully aquatic hosts (Poulin 1992, Dusek et al. 1998, MacKenzie 1999, Huspeni and Lafferty 2004, Marcogliese 2005, Perez-del Olmo et al. 2007, Oluoch-Otiego et al. 2016). Furthermore, few studies have investigated how trace elements interact with parasites of terrestrial or semi-aquatic mammals (Klenavic et al. 2008, McGrew et al. 2015, Binkowski et al. 2016, Hernandez et al. 2017). Semi-aquatic mammals such as the North American river otter (*Lontra canadensis*), North American beaver (*Castor canadensis*), and raccoon (*Procyon lotor*) make good model species for ecotoxicology studies as they can move freely between contaminated aquatic and terrestrial systems, transporting contaminants between the two. These species also represent multiple trophic levels at which toxicological effects can be assessed (Klenavic et al. 2008, Lotze and Anderson 1979, Novak 1987). A previous study found that raccoons captured in a wetland habitat contaminated with trace elements had a greater abundance and diversity of parasites than raccoons from an uncontaminated site (Hernandez et al. 2017). However, it is possible such differences in parasite diversity and abundance could be due to differences in biotic or abiotic factors among sampling areas, rather than trace element contamination. Additionally, there is a general lack of recent contaminant data for furbearers in the Southeast United States (Jenkins et al. 1969; Cumbie and Jenkins 1974, Cumbie 1975a, 1975b; Carmichael and Baker 1989).

Our study seeks to assess the exposure of river otters, beavers, and raccoons in the Southeast United States, including the U.S. Department of Energy's Savannah River Site (SRS), to trace elements and ^{137}Cs contamination, and the potential effects of contaminant exposure on their endoparasite communities. We hypothesized that: 1) animals sampled from the SRS would have elevated levels of trace elements and ^{137}Cs compared to reference sites in South Carolina and Georgia; 2) river otters would have the highest mercury concentrations, raccoons would have intermediate concentrations, and beavers would have the lowest concentrations due to differences in trophic position and the propensity for mercury to biomagnify within food webs; and 3) animals with higher contaminant concentrations would have higher macro-endoparasite abundance due to potentially decreased immune response and lower parasite diversity due to the lack of intermediate hosts in contaminated systems.

METHODS

Study Area

This research was conducted on the SRS, 10 counties in Georgia (Burke, Catoosa, Clarke, Cook, Elbert, Gwinnett, Morgan, Murray, Oconee, and Oglethorpe) and 11 counties in South Carolina (Calhoun, Chester, Clarendon, Dillon, Florence, Kershaw, McCormick, Richland, Sumter, Williamsburg, and York), USA. The SRS is a limited access Department of Energy (DOE) facility, located near Aiken, SC. Established in 1951 by the Atomic Energy Commission as a nuclear production facility, the SRS covers a 780 km^2 area in the upper Coastal Plain of South Carolina (White and Gaines 2000). The SRS is a mosaic of industrial activity and forested land, 64% of which is upland pine forest and 15% is bottomland hardwood forest, containing abundant streams, marshes, and artificial impoundments (White and Gaines 2000, DeVault et al. 2004).

Numerous waterbodies on the SRS have been affected by facility operations and have received inputs of trace elements and radionuclides. In particular, extensive trace element contamination now exists within the D-Area ash basins, which received sluiced fly and bottom ash containing (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Se, and Zn) from 1953-2012 (Gutherie and Cherry 1979, Rowe et al. 1996). Other areas on the SRS where trace elements are a concern include A-01 constructed wetlands, Tim's Branch/Steed's Pond, and Fourmile Branch (Kvartek et al. 1994, Nelson et al. 2002, Edwards et al. 2014).

Additionally, there are four main areas on the SRS where wildlife has the potential to be exposed to ^{137}Cs: Fourmile Branch, Steel Creek, Lower Three Runs Creek, and Pen Branch (Carlton et al. 1992). Of these, Lower Three Runs Creek ($8.2x10^{12}$ Bq) and Fourmile Branch ($1.2x10^{12}$ Bq) received the most extensive inputs of ^{137}Cs during the operational lifetime of R and C reactors, respectively (Carlton et al. 1992). Steel Creek received $1.1x10^{12}$ Bq of ^{137}Cs from L Reactor while Pen Branch $0.9x10^{12}$ Bq from K Reactor until they were shut down in 1988 (Carlton et al. 1992). Given the mix of forested habitat, industrial areas, uncontaminated, and contaminated sites, the SRS represents an ideal landscape to study the effects of environmental contaminants on wildlife, and numerous studies have reported elevated contaminant concentrations in various organisms inhabiting the SRS (Burger et al. 2002, Edwards et al. 2014, Oldenkamp et al. 2017).

The majority of our non-SRS study sites were located in the Piedmont and Coastal Plain regions of SC and GA. The Piedmont consists of foot hills characterized by acid crystalline and metamorphic rock and receives 112 to 142cm of mean annual rainfall (Turner and Ruscher 1988). Predominant forest types of the Piedmont region are loblolly-short leaf pine and oak-pine forests (Turner and Ruscher 1988). Areas of the coastal plain have gentle to moderate slopes and have loam and/or clay soils (Turner and Ruscher 1988). Dominant forest types in the Coastal Plain are longleaf-slash pine and loblolly-shortleaf pine with oak-gum-cypress occurring along rivers and flood plains (Turner and Ruscher 1988). Mean annual rainfall in the Coastal Plain ranges from 112 and 135cm (Turner and Ruscher 1988). Of our counties in South Carolina, Calhoun, Clarendon, Dillon, Florence, Kershaw, Richland, Sumter, and Williamsburg occurred in the Coastal Plain, while Chester, McCormick, and York occurred in the Piedmont. In Georgia, Burke, Clarke, Elbert, Gwinnet, Morgan, Oconee, and Oglethorpe occurred in the Piedmont, Catoosa occurred in the Ridge, Valley and Cooke occurred in the Coastal Plain, and Murray occurred in the Blue Ridge.

Field Methods

Otters, beavers, and raccoons were trapped during January 2013 to November 2018 from various locations throughout the SRS (including contaminated waterbodies) and throughout Georgia and South Carolina. SRS sampling locations with known contamination included Steel Creek, A-01 Constructed Wetlands, the R-Canal/Pond A/Pond B complex, Steed's Pond/Tim's Branch, and Fourmile Branch (Gutherie and Cherry 1979, Carlton et al. 1992, Kvartek et al. 1994, Rowe et al. 1996, Nelson et al. 2002, Edwards et al. 2014). Additional SRS samples were collected from uncontaminated sites including Upper Three Runs Creek and Fire Pond. Reference sites outside of the SRS in GA included Burke, Catoosa, Clarke, Cook, Elbert, Gwinnett, Morgan, Murray, Oconee, and Oglethorpe counties. Reference sites in SC included Calhoun, Chester, Clarendon, Dillon, Florence, Kershaw, McCormick, Richland, Sumter, Williamsburg, and York counties.

River otters and beavers were trapped using body grip traps (#330 Body Grip Trap; Duke Company, West Point, MS) set in stream channels and beaver dam cross-overs with the springs set below water. Otters were also targeted at latrine sites using foot-hold traps (MB-550-RJ; Minnesota Trapline Products, Pennock, MN). Raccoons were captured using box live traps placed in close proximity to roads and waterbodies (Tomahawk No. 108; Tomahawk Live Trap Co., Tomahawk, WI). Upon capture on the SRS, animals caught in foot-hold and box traps were euthanized according to American Association of Wildlife Veterinarian guidelines via gunshot (AVMA 2013). Additional reference animals were obtained from non-SRS locations in South Carolina and Georgia through the United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA-APHIS). All animals were assigned a unique identification number and the capture location and species was recorded. All carcasses were frozen and then brought to the University of Georgia, Savannah River Ecology Laboratory until necropsies could be performed. All animal handling practices and euthanasia were carried out with accompanying state collecting permits, and individuals collected on the SRS were captured and euthanized in accordance with the University of Georgia Animal Care and Use Guidelines under Animal Care and Use Protocol A2015 12-017-Y3-A2.

Laboratory Methods

<u>*Necropsies*</u>

Carcasses were thawed and necropsied to collect sex, age, morphometric measurements, and parasite data, as well as liver and muscle tissue samples to be used for trace element and ^{137}Cs analysis, respectively. Morphometric data included weight, total length, tail length, chest girth, and hind foot length. Intestinal helminths were sampled by opening the stomach and splitting the intestines longitudinally with scissors and examining the contents. Stomach and intestinal contents were first visually inspected where we removed any grossly visible macroparasites; the contents were then rinsed through a series of sieves ranging from 4,000 µm – 300 µm to optimize detection of remaining grossly visible macroparasites. All parasites were preserved in 70% ethanol and identified to genus (species when possible) using stereomicroscopy. Liver and muscle tissue samples were placed in labeled polyethylene Whirl-Pak bags, frozen, freeze dried, and homogenized for subsequent trace element and ^{137}Cs analysis.

Trace Element Analysis

We analyzed the concentrations of 10 trace elements: Hg, Cr, Ni, Cu, Zn, As, Se, Cd, Pb, and U. For the analysis of Cr, Ni, Cu, Zn, As, Se, Cd, Pb, and U, 250 mg of homogenized liver sample, certified reference material (TORT-3; National Research Council, Ottawa, ON, Canada), blanks, and a duplicate for every 20 samples were microwave digested (MARSX Xpress, CEM Corporation, Matthews, NC) with 10.0 ml of 70% nitric acid. Certified reference material and blanks were used to obtain percent recoveries and as positive and negative controls, respectively.

After acid digestion, the sample was transferred to a 15 ml tube and brought to a volume of 15 ml by adding 5 ml of Milli-Q purified water (EMD Millipore, Merc KGaA, Darmstadt, Germany). Samples were analyzed using inductively coupled plasma mass spectroscopy (Nexlon 300X ICP-MS; Perkins Elmer, Norwalk, CT, USA) with a 1:10 dilution factor (1.0 ml sample to 9.0 ml Milli-Q water). We obtained the method detection limits (MDL) in ppm for each element on a dry mass basis. Due to differences between runs, MDL values are reported as $\bar{x} \pm$ SE: Cr (0.175±0.060), Ni (0.183±0.110), Cu (0.158±0.086), Zn (1.016±0.696), As (0.143±0.034), Se (0.814±0.324), Cd (0.080±0.016), Pb (0.059±0.016), U (0.085±0.022). Mean percent recoveries ranged from 85-105% for elements in certified reference material and are presented in ppm on a dry mass basis. Concentrations for duplicate samples are presented as means.

Total mercury (THg), hereafter mercury (Hg), was analyzed using 10-15 mg of liver sample for direct mercury analysis (DMA80; Milestone, Shelton, CT, USA). According to EPA method 7473 (EPA 2007). Within each set of 10 samples, a duplicate, blank, and two standard reference materials were used (TORT-3 and PACS-2, National

Research Council of Canada, Ottawa, ON) as quality assurance controls. Method detection limit averaged 0.00146 ppm on a dry mass basis. Percent recoveries in certified reference material ranged from 89-132%. Duplicate samples were typically within 20% of one another. If duplicates fell beyond this range a triplicate sample was analyzed. Means of replicate samples were used in analyses.

We quantified ^{137}Cs activity within muscle tissue using a Packard Cobra II Auto-Gamma Counter (Model Cobra II 5003) with a single 3-inch through-hole NaI detector that was auto-calibrated using a traceable sealed source of ^{137}Cs (SREL sealed source 0113). Homogenized muscle tissue samples were packed into 5 ml polystyrene tubes with weights ranging from 0.5 – 0.2 g for counting efficiency. The Auto-Gamma counter was set for ^{137}Cs emission centered at 662 kiloelectron-volts (keV) photons with a counting region of interest between 580-754 keV to absorb and record ^{137}Cs emissions. Samples were analyzed for 3600 count intervals, with background samples at every fifth sample position.

Statistical Analysis

We compared trace element and ^{137}Cs concentrations in otters, beavers, and raccoons from the SRS, SC, and GA, and examined differences among species and locations. All statistical analyses were conducted using R statistical software (version 3.3.3, The R Foundation for Statistical Computing 2017). We excluded any elements from analyses where >50% of samples were below the MDL for any one species. For the remaining elements we replaced concentrations that were below MDL with 50% of the respective MDL of that element (Hall et al. 2009, Fletcher et al. 2014). We used a Spearman's rank correlation to test for collinearity between the remaining elements, and excluded any variables where $|r_s| > 0.70$. We tested all element distributions for normality using a Shapiro-Wilk test and subsequently natural log-transformed element concentration data to approximate normality prior to analysis. We calculated the percent moisture in muscle and liver samples for all three species and found that mean percent moisture in liver tissue was 70.9%, 70.4%, and 66.6% for beavers, otter, and raccoons, respectively. Due to the similarity in percent moisture in the livers of all three species, we pooled these moisture values and used the mean percent moisture (69.3%) for all three species. We used this to calculate wet weight concentrations of each element for liver samples. We found that percent moisture in muscle tissue was 74.8%, 73.2%, and 74.0% for beavers, otters, and raccoons, respectively. Again, due to the similarity we calculated the mean percent moisture as 74.0% for all three species. In text and figures, concentrations are reported as dry weight (d.w.), unless stated otherwise. For ease of comparison to other studies we report concentrations for d.w. in Tables 2.1-2.3 and concentrations for wet weight (w.w.) in Tables 2.4-2.6.

We observed ^{137}Cs concentrations in some muscle samples that were below background radiation levels, resulting in negative values. Negative values and small positive values below minimum detectable concentrations often are not reported or set to an indicator value, which biases the mean and variance of the data (Gilbert and Kinnison 1981, Newman et al. 1989). For this reason, we included all negative values and those below minimum detectable concentration when reporting ^{137}Cs concentrations and performing analyses. These data were non-normally distributed; therefore, they were scaled by adding one and log transformed prior to analysis.

We used a multivariate analysis of variance (MANOVA) model with the Pillai-Bartlett statistic to test whether there was a difference in element concentrations among species, locations, and the interaction between these variables. We then conducted *post hoc* two-way analysis of variance (ANOVA) with Tukey's HSD test to make comparisons between species and locations.

We used negative binomial generalized linear mixed effect models to investigate how parasite abundance and species richness are influence by trace element concentrations in their host, using abundance and species richness as dependent variables in separate models. Competing models were compared using Akaike information criterion (AIC), and considered all models within $\Delta 2$ AIC competitive (Burnham and Anderson 2004). For raccoons, we included site as a random effect within our model, as well as a set of six metals known to be important to wildlife health or have previously been shown to have effects on parasites as fixed effects: Cu, Zn, Cd, Pb, Se, and Hg (Poulin 1992, Govind and Madhuri 2014, Hernandez et al. 2017). A similar model was constructed for beaver, although Se was not included in the model because >50% of samples had Se concentrations below the method detection limit. We did not find a sufficient number of parasites in otters to make statistical comparisons (see results). Metal concentrations were natural log-transformed prior to analysis due to non-normal distributions. Due to the difficulty of accurately quantifying cestode abundance, if a cestode taxa was present it was counted as one.

RESULTS

Locational and Species Differences

We collected 62 beavers (GA = 31, SC = 11, SRS = 20), 50 otters (GA = 15, SC = 26, SRS = 9), and 86 raccoons (GA = 31, SC = 8, SRS = 47) between January 2013 and November 2018. Of the elements analyzed, 6 had >50% of samples above the method detection limit for all three species and were included in subsequent analyses: Cr, Cu, Zn, Cd, Hg, and ^{137}Cs. For Ni, only beavers had ≥50% of samples >MDL (Table 2.1-2.3). For As, only raccoons had ≥50% of samples >MDL (Table 2.1-2.3). Otters and raccoons had ≥50% of samples >MDL for As and SE, but beavers did not (Table 2.1-2.3). Over 50% of samples were <MDL for U for all three species (Table 2.1-2.3). Spearman's rank correlation tests indicated all other elements had correlation values $|r_s| < 0.70;$ thus all were included in subsequent models.

The results of our MANOVA revealed there was a significant difference in element concentrations among species ($F_{2,188} = 63.60, P < 0.001$) and locations ($F_{2,188} = 7.73\ P < 0.001$), the interaction term was also significant in the model ($F_{4,188} = 2.58\ P < 0.001$). For Cr, no significant difference was observed between river otters, beavers, and raccoons collected from South Carolina and Georgia. However, raccoons and beavers collected from the SRS contained significantly higher Cr concentrations than GA and SC samples, but were not significantly different from each other (Figure 2.1). Across all three sampling sites, hepatic Cd concentrations were elevated in raccoons but were only significantly higher than beaver and otter in GA, beaver in SC, and beaver on the SRS (Figure 2.2). The highest hepatic Cu concentrations were found in raccoons from SC (geometric $\bar{x} = 156.35 \pm 57.07$ppm; Table 2.3), but concentrations did not differ statistically from raccoons sampled in GA, or from otters in SC and on the SRS (Figure 2.3). South Carolina raccoons had significantly higher Cu concentrations than raccoons from the SRS and otters from Georgia (Figure 2.3). Hepatic Cu concentrations in beavers were lower than concentrations in raccoons and otters, regardless of sampling location (Figure 2.3). The highest hepatic Zn concentrations were found in raccoons from Georgia (geometric $\bar{x} = 147.31 \pm 7.27$ppm; Table 2.3), which were significantly higher than concentrations found in otters and beavers across all locations, and raccoons from the SRS, but did not differ from raccoons sampled in SC (Figure 2.4). Otters on the SRS had the highest observed hepatic Hg concentration (50.90 ppm), and Hg concentrations were generally higher in otters from the SRS (geometric $\bar{x} = 15.15 \pm 5.55$ppm; Table 2.2). However, Hg concentrations in SRS otters were not significantly greater than otters from SC or GA, or raccoons from the SRS (Figure 2.5). Hepatic Hg concentrations in beaver were lower than otters and raccoons across all of our sampling locations (Figure 2.5). We found concentrations of ^{137}Cs in muscle tissue to be slightly elevated for individuals sampled on the SRS, particularly raccoons and river otter. However, ^{137}Cs concentrations did not differ among species or sampling locations, with the exception that concentrations in raccoons were higher than concentrations in beaver from GA and SC, but not the SRS (Figure 2.6).

Parasite Abundance and Diversity

We found seven helminth taxa in raccoons from GA, SC, and the SRS: one acanthocephalan (*Macracanthorhynchus ingens*), one cestode (*Atriotaenia* sp.), and five nematodes (*Placoconus lotoris*, *Gnathostoma procyonis*, *Physaloptera* sp., *Molineus barbatus*, and *Baylisascaris procyonis*). An average of 1.49±0.17 taxa of helminths were found in raccoons from GA, 0.875±0.35 helminth taxa were found in raccoons from SC, and 1.83±0.14 helminth taxa were found in raccoons from the SRS (Table 2.7). Average total abundance was 5.03±1.23 parasites in GA raccoons, 10.25±6.9 parasites in SC raccoons, and 18.49±3.19 parasites in SRS raccoons (Table 2.7). Only one binomial mixed model for species richness was supported and showed that Se concentration was an important factor describing the species richness of parasites in raccoons (Intercept: $\beta_1 = 0.05, P =$ 0.76; ln(Se ppm): $\beta_2 = 0.23, P = 0.014$); richness was greatest in raccoons from the SRS (Table 2.7). One negative binomial mixed model describing total abundance of parasites in raccoons was supported and showed that Hg concentration was important in describing total parasite abundance in raccoons (Intercept: $\beta_1 = 2.13, P < 0.01$; ln(Hg ppm): $\beta_2 = 0.23, P = 0.060$), however Hg concentrations were not significant.

One species of parasite was found in beavers from GA, SC, and the SRS, the trematode *Stichorchis subtriquetrus*. Average *S. subtriquetrus* abundance was 16.68±4.82 in beavers from GA, 32.5±12.64 in beavers from SC, and 21.2±5.56 in beavers from the SRS (Table 2.8). The only supported negative binomial mixed model for *S. subtriquetrus* abundance indicated that Hg concentration influenced the abundance of *S. subtriquetrus*, with abundance increasing with Hg concentration (Intercept: $\beta_1 = 1.28, P =< 0.01$; ln(Hg ppm): $\beta_2 = 0.82, P = 0.030$). Out of the 50 otters sampled, we found 6 to have nematode parasites and 1 to have trematode parasites. We did not find enough parasites in otters to be deemed useful for analysis. Otter parasites were preserved but not identified.

DISCUSSION

Our study is the first to directly compare trace element concentrations among semi-aquatic mammals of varying trophic levels in the Southeastern United States, where elevated Hg levels are pervasive throughout many aquatic environments (Burger et al. 2002, Cumbie and Jenkins 1974, Wren 1984). As hypothesized, our data revealed the biomagnification of Hg as a function of trophic position, with lowest concentrations in beavers, intermediate concentrations in raccoons, and highest concentrations in river otters. However, our maximum concentration of hepatic Hg of 15.63 ppm w.w. was below the threshold of 33 ppm w.w. associated with lethality (O'Connor and Nielsen 1980). While tissue concentration data for sublethal effects in otters is lacking, hepatic Hg concentrations of 30 ppm w.w. of in mink (*Neovison vison*) have been associated with impaired reproduction (Wren 1987).

In addition to trophic level differences in Hg concentrations, spatial variation in Hg exposure can occur due to numerous biotic and abiotic factors (Wren 1986). Among otters, mean hepatic Hg concentrations were highest in individuals collected on the SRS (15.15 ppm d.w., 4.65 ppm w.w.) and in South Carolina (12.49 ppm d.w, 3.83 ppm w.w.), both of which were higher than values reported throughout much of the northern U.S. and Canada (range: 1.7-3.3 ppm w.w., 6.8-12.0 ppm d.w.; Sheffy and St. Amant 1982, Wren 1986, Klenavic et al. 2008, Stansley et al. 2010, Mayack 2012). Interestingly, otters collected from Georgia had lower Hg concentrations than our other sampling sites, and substantially lower concentrations than values reported previously in other parts of Georgia (Ware County 9.16ppm w.w., Echols County 5.11ppm w.w.; Halbrook et al. 1994). This locational variation is likely attributed to differing soil types among sites, as within Georgia the majority of our animals were collected in the Piedmont region, while most of our animals from South Carolina were collected within the Upper and Lower Coastal Plain. Mercury is generally more bioavailable to soil organisms within the Coastal Plain, due to soils being more acidic and poorly drained, and having a smaller pool of available nutrients and low ion exchange capacity (Cumbie and Jenkins 1974, Harris 1971). Similarly, Cumbie and Jenkins (1974) compared Hg concentrations in bobcats (*Lynx rufus*), raccoons, Virginia opossums (*Didelphis virginiana*), and gray fox (*Urocyon cinereoargenteus*) along a transitional gradient from the Piedmont of Georgia through the Upper and Lower Coastal Plain, including the SRS. Their study revealed Hg concentrations in mammals was lowest in the Piedmont, and increased with closer proximity to the coast, with the highest concentrations on the SRS (Cumbie and Jenkins1974).

While there were no apparent trends in hepatic concentrations of Cr, Cu, Zn, and ^{137}Cs among species, Cd concentrations were consistently higher in raccoons than beaver and river otter across all locations. However, Cd concentrations in raccoons were still well below the concentration (10 ppm w.w assuming 60% moisture) associated with overtly toxic contamination in mammals (Wren 1995). The difference in hepatic Cd between raccoons, beavers, and otters may be due to the biomagnification of Cd within terrestrial food webs (Mayack 2012). A substantial portion Cd is stored in plant biomass within terrestrial ecosystems and has been shown to bioaccumulate in terrestrial herbivores (Larison et al. 2000, Nolet et al. 1994, Mayack et al. 2012). This is a reasonable explanation for the difference in hepatic Cd concentrations between raccoons and otters, considering raccoons are opportunistic omnivores that feed in both terrestrial and aquatic systems, while otters primarily consume fish and invertebrates in aquatic systems (Barding and Lacki 2012, Rulison et al. 2012). Although beavers consume terrestrial vegetation, they may not have exhibited elevated cadmium due to their low trophic position relative to raccoons.

The SRS has a history of environmental contamination, and thus we expected wildlife sampled from the SRS to have elevated concentrations of trace elements and radionuclides known to be present on the site (i.e. ^{137}Cs, Hg, Se) compared to other sampling locations (Burger et al. 2002, Oldenkamp et al. 2017). However, among the elements evaluated in our study, only Cr concentrations in raccoons and beavers on the SRS were consistently higher than those of individuals collected off-site, and no differences in Cr concentrations were observed in otters among locations. Further, our findings for Cr in raccoons were similar to those previously reported in raccoons on the SRS and were at concentrations below that which would be associated with negative health effects (Burger et al. 2000, Hernandez et al. 2017). The lack of evidence for elevated trace element concentrations in SRS furbearers compared to the surrounding landscape likely reflects the fact that most trace elements introduced into the SRS landscape are highly localized and associated with specific industrial activities (e.g., D-area coal ash basins). Although some individuals in our study were collected from SRS locations with known trace element inputs, these individuals were able to freely move between contaminated and uncontaminated areas, and samples were also collected from areas of no extensive trace element inputs. Thus, while previous studies evaluating contaminant uptake in wildlife inhabiting localized contaminated environments on the SRS have demonstrated increased concentrations of various elements (Hernandez et al.

2017, Oldenkamp et al. 2017), our results suggest contaminant concentrations in wildlife collected broadly across the SRS are generally similar to those collected from non-SRS locations in the surrounding landscape.

Surprisingly, muscle concentrations of ^{137}Cs did not differ significantly between the SRS and our other sampling locations, despite the known presence of this radionuclide on the SRS. However, across all species, and particularly for raccoons and otters, there was a general trend of elevated ^{137}Cs concentrations among individuals sampled on the SRS. The lack of a significant difference among our sampling sites was likely due to the fact that while we did catch individuals in known ^{137}Cs contaminated areas, the majority of SRS animals were captured from areas of the SRS with no known ^{137}Cs inputs from SRS activities. This is reflected by the wide variance in ^{137}Cs concentrations among individuals (especially raccoons) sampled on the SRS compared to other sampling locations. Further, the distribution of ^{137}Cs on the SRS is primarily restricted to a handful of aquatic systems. Given the home range sizes of otter and beaver (Helon et al. 2004, McClintic et al. 2014), and ability of raccoons to forage in terrestrial (non-^{137}Cs contaminated locations) systems, ^{137}Cs concentrations observed on the SRS likely reflect spatial and temporal heterogeneity in exposure to this contaminant, even for individuals sampled from areas with known ^{137}Cs contamination.

Our results indicate that elevated Hg concentrations in beaver were associated with an increase in abundance of *S. subtriquetrus*, and that hepatic Hg concentration was positively associated with the total abundance of parasites in raccoons. However, Hg concentrations in beaver were generally very low and the effect of Hg concentrations in raccoons was not significant, thus the implications of this finding are unclear. While previous studies have linked the death of Great White Herons (*Ardea herodias occidentalis*) to chronic disease and elevated Hg concentrations (Spalding et al. 1994), and infection has been found to increase in relation to Hg in harbor porpoises (*Phocoena phocoena*) (Bennett et al. 2001), the dose-dependent response between Hg concentration and immune function in wildlife requires further study (Scheuhammer et al. 2007). Klenavic et al. (2008) found that mink infected with *Dioctophyma renale* had higher Hg concentrations than uninfected mink, but the mechanism for this finding is unknown.

Species richness of parasites in raccoons also increased relative to hepatic Se concentrations. This result was unexpected, as Se is known to have lethal toxic effects on parasites (Riggs et al. 1987). Previously, Hernandez et al. (2017) found that raccoons with higher Cu concentrations on the SRS had a higher abundance of intestinal helminths. While Cu concentrations in raccoons sampled in our study were similar to those reported in Hernandez et al. (2017), Cu was not an important predictor of raccoon endoparasite abundance in our analyses. Such differences among studies highlights the complexity associated with host-parasite-contaminant interactions among free-ranging wildlife populations. In particular, parasite abundance and distribution can differ between sampling locations due to a suite of biotic and abiotic factors, irrespective of contaminant inputs (Bafundo et al. 1980).

Concentrations of trace elements can vary greatly among individuals, organisms, and ecosystems, driven by a variety of biotic and abiotic factors. For example, the type and source of anthropogenic pollution, soil properties, the trophic position and natural history of the organism, and the chemical properties of the element in question all interact to contribute to observed contaminant concentrations within biota (Chase et al. 2001, Rattner and Heath 2003, Wren et al. 1984). Differences in trace element concentrations between geographic locations can often be explained by proximity to sources of anthropogenic contamination and the abiotic and biotic factors of the ecosystem, while differences in trace elements between species are best explained by differences in diet and natural history (Barron 2003, Rattner and Heath 2003). Our findings reflect the inherent variability in the distribution and bioavailability of anthropogenic contaminants throughout the landscape, and exemplify the importance of broad-scale sampling when assessing general trends in contaminant burdens within species. We also note that Hg and Se concentrations in animals may influence their endoparasite communities, however the mechanisms contributing to these patterns need further investigation. Future research also is needed to elucidate the sublethal effects of Hg in river otter, particularly in areas of the southeastern U.S. or other areas where anthropogenic inputs of Hg have been pervasive.

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Table 2.1: Comparison of trace element concentrations (ppm, d.w.) in liver tissue and ^{137}Cs (Bq/g, d.w.) in muscle tissue of beavers from Georgia (GA), South Carolina (SC), and the Savannah River Site (SRS), USA, 2013-2018, (—) designates value <MDL.

	GA (N=31)			SC (N=11)			SRS (N=20)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Cr	0.16	0.03	0.06 – 0.69	—	—	—	0.68	0.067	0.16 – 1.08
Ni	—	—	—	—	—	—	0.11	0.02	0.01 – 0.40
Cu	20.18	7.23	4.93 – 223.59	9.56	0.45	6.37 – 11.44	10.79	0.72	7.56 – 20.41
Zn	111.50	3.32	78.17 – 150.35	106.42	6.52	71.42 – 133.48	106.42	6.52	71.42 – 133.48
As	—	—	—	—	—	—	—	—	—
Se	—	—	—	—	—	—	1.05	0.12	0.36 –2.82
Cd	0.36	0.07	0.06 – 1.51	0.33	0.10	0.06 – 1.13	0.40	0.09	0.04 – 1.73
Pb	—	—	—	—	—	—	0.06	0.02	0.02 – 0.31
U	—	—	—	—	—	—	—	—	—
Hg	0.04	0.01	0.01 – 0.19	0.04	0.00	0.01 – 0.06	0.05	0.00	0.02 – 0.09
^{137}Cs	0.01	0.01	-0.07 – 0.01	0.05	0.0374	-0.09 – 0.39	0.08	0.02	-0.20 – 0.29

Table 2.2: Comparison of trace element concentrations (ppm, d.w.) in liver tissue and ^{137}Cs (Bq/g, d.w.) in muscle tissue of otters from Georgia (GA), South Carolina (SC), and the Savannah River Site (SRS), USA, 2013-2018, (—) designates value <MDL.

	GA (N=15)			SC (N=26)			SRS (N=8)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Cr	—	—	—	0.21	0.04	0.06 – 0.10	—	—	—
Ni	—	—	—	—	—	—	—	—	—
Cu	33.59	7.40	9.43 – 130.85	45.94	11.63	15.10 – 322.69	52.67	18.52	15.09 –171.13
Zn	90.95	4.07	63.12 – 126.46	91.00	3.90	59.16 – 130.76	91.00	3.90	59.16 – 130.76
As	—	—	—	—	—	—	—	—	—
Se	4.06	0.36	1.96 – 6.32	4.34	0.38	0.36 – 8.68	5.57	1.51	3.25 – 15.74
Cd	0.24	0.03	0.06 – 0.50	0.34	0.05	0.13 – 1.42	0.74	0.18	0.06 – 1.35
Pb	0.21	0.08	0.03 – 1.17	0.23	0.04	0.03 – 0.69	0.46	0.21	0.03 – 1.46
U	—	—	—	—	—	—	—	—	—
Hg	4.78	0.60	1.25 – 9.86	12.49	1.08	1.77 – 20.02	15.15	5.55	0.43 – 50.90
^{137}Cs	0.03	0.01	-0.04 – 0.10	0.00	0.01	-0.12 – 0.09	0.20	0.07	0.02 – 0.59

Table 2.3: Comparison of trace element concentrations (ppm, d.w.) in liver tissue and ^{137}Cs (Bq/g, d.w.) in muscle tissue of raccoons from Georgia (GA), South Carolina (SC), and the Savannah River Site (SRS), USA, 2013-2018, (—) designates value <MDL.

	GA (N=31)			SC (N=8)			SRS (N=47)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Cr	0.25	0.05	0.06 – 1.23	0.16	0.04	0.06 – 0.38	0.79	0.14	0.06 – 4.48
Ni	—	—	—	0.15	0.03	0.06 – 0.34	0.43	0.08	0.05 – 2.29
Cu	37.05	3.62	13.10 – 109.56	156.35	57.07	11.01 – 407.17	34.44	2.56	14.26 – 88.66
Zn	147.31	7.27	93.77 – 244.47	130.94	9.66	92.40 – 166.35	130.94	9.66	92.40 – 166.35
As	0.11	0.01	0.04 – 0.25	0.04	0.01	0.04 – 0.07	0.26	0.03	0.04 – 1.00
Se	2.56	0.23	0.36 – 4.44	2.40	0.92	0.36 – 6.82	5.54	0.43	0.71 – 16.28
Cd	0.83	0.18	0.12 – 5.05	1.11	0.34	0.16 – 3.10	1.27	0.17	0.06 – 5.50
Pb	0.43	0.16	0.02 – 4.82	2.66	1.07	0.66 – 9.73	0.53	0.11	0.09 – 4.53
U	—	—	—	—	—	—	—	—	—
Hg	1.82	0.28	0.12 – 5.67	4.67	2.71	0.09 – 22.64	4.32	0.49	0.39 – 13.42
^{137}Cs	0.07	0.01	-0.08 – 0.23	0.06	0.04	-0.04 – 0.29	0.28	0.09	-0.08 – 2.89

Table 2.4: Comparison of trace element concentrations (ppm, w.w.) in liver tissue and ^{137}Cs in muscle tissue (Bq/g, w.w.) of beavers from Georgia (GA), South Carolina (SC), and the Savannah River Site (SRS), USA, 2013-2018.

	GA (N=31)			SC (N=11)			SRS (N=20)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Cr	0.05	0.01	0.02 – 0.21	—	—	—	0.21	0.02	0.05 – 0.33
Ni	—	—	—	—	—	—	0.03	0.01	0.00 – 0.12
Cu	6.19	2.22	1.51 – 68.64	0.14	0.14	1.96 – 3.51	3.31	0.22	2.32 – 6.27
Zn	34.23	1.02	24.00 – 46.16	32.67	2.00	21.93 – 40.98	32.67	2.00	21.93 – 40.98
As	—	—	—	—	—	—	—	—	—
Se	—	—	—	—	—	—	0.32	0.04	0.11 – 0.87
Cd	0.11	0.02	0.02 – 0.46	0.10	0.03	0.02 – 0.35	0.12	0.03	0.01 – 0.53
Pb	—	—	—	—	—	—	0.02	0.00	0.01 – 0.09
U	—	—	—	—	—	—	—	—	—
Hg	0.01	0.00	0.00 – 0.06	0.01	0.00	0.00 – 0.02	0.01	0.00	0.01 – 0.03
^{137}Cs	0.00	0.00	-0.02 – 0.02	0.01	0.01	-0.02 – 0.10	0.02	0.01	-0.05 – 0.08

Table 2.5: Comparison of trace element concentrations (ppm, w.w.) in liver tissue and ^{137}Cs (Bq/g, w.w.) in muscle tissue of otters from Georgia (GA), South Carolina (SC), and the Savannah River Site (SRS), USA, 2013-2018, (—) designates value <MDL.

	GA (N=15)			SC (N=26)			SRS (N=8)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Cr	—	—	—	0.07	0.01	0.02 – 0.31	—	—	—
Ni	—	—	—	—	—	—	—	—	—
Cu	10.31	2.27	2.90 – 40.17	14.10	3.57	4.64 – 99.07	16.17	5.69	4.63 – 52.54
Zn	27.92	1.25	19.38 – 38.82	27.94	1.20	18.16 – 40.14	27.94	1.20	18.16 – 40.14
As	—	—	—	—	—	—	—	—	—
Se	1.25	0.11	0.60 – 1.94	1.33	0.12	0.11 –2.66	1.71	0.46	0.10 – 4.83
Cd	0.07	0.01	0.02–0.15	0.10	0.02	0.04 – 0.44	0.23	0.05	0.02 – 0.41
Pb	0.06	0.02	0.01 - 0.36	0.07	0.01	0.01 – 0.21	0.14	0.06	0.01 – 0.45
U	—	—	—	—	—	—	—	—	—
Hg	1.47	0.18	0.38 – 3.03	3.83	0.33	0.54 – 6.14	4.65	1.70	0.13 – 15.63
^{137}Cs	0.01	0.00	-0.01 – 0.03	0.00	0.00	-0.03 – 0.02	0.05	0.02	0.00 – 0.15

Table 2.6: Comparison of trace element concentrations (ppm, w.w.) in liver tissue and ^{137}Cs (Bq/g, w.w.) in muscle tissue of raccoons from Georgia (GA), South Carolina (SC), and the Savannah River Site (SRS), USA, 2013-2018, (—) designates value <MDL.

	GA (N=31)			SC (N=8)			SRS (N=47)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Cr	0.08	0.02	0.02 – 0.38	0.05	0.01	0.02 – 0.12	0.24	0.04	0.02 – 1.37
Ni	—	—	—	0.05	0.01	0.02 – 0.10	0.13	0.03	0.02 – 0.70
Cu	11.37	1.11	4.02 – 33.63	48.00	17.52	3.38 – 125.00	10.57	0.79	4.38 – 27.22
Zn	45.23	2.23	28.79 – 75.05	40.20	2.69	28.37 – 51.07	40.20	2.96	28.37 – 51.07
As	0.03	0.00	0.01 – 0.08	0.01	0.00	0.00 – 0.02	0.08	0.01	0.01 – 0.31
Se	0.79	0.07	0.11 – 1.36	0.74	0.28	0.12 – 2.09	1.70	0.13	0.22 – 5.00
Cd	0.26	0.06	0.04 – 1.55	0.34	0.11	0.05 – 0.95	0.39	0.05	0.02 – 1.69
Pb	0.13	0.05	0.01 – 1.48	0.82	0.33	0.20 – 3.00	0.16	0.03	0.03 – 1.39
U	—	—	—	—	—	—	—	—	—
Hg	0.56	0.08	0.03 – 1.74	1.42	0.83	0.03 – 6.95	1.33	0.15	0.12 – 4.12
^{137}Cs	0.02	0.00	-0.02 – 0.06	0.01	0.01	-0.01 – 0.07	0.07	0.02	-0.02 –0.75

Table 2.7: Species richness and abundance of parasites in raccoons collected from Georgia (GA), South Carolina (SC), and the U.S. Department of Energy's Savannah River Site (SRS), USA, 2013-2018.

	GA (N=31)			SC (N=8)			SRS (N=47)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Species Richness	1.49	0.17	0 – 4	0.88	0.35	0 – 2	1.83	0.14	0 – 4
Total Abundance	5.03	1.23	0 – 28	10.25	6.9	0 – 57	18.49	3.19	0 – 86
Macracanthorhynchus ingens	3.39	0.92	0 – 19	2.50	1.22	0 – 9	8.30	1.44	0 – 37
Atriotaenia sp.	0.26	0.08	0 – 1	0	-	-	0.19	0.06	0 – 1
Placoconus lotoris	0.77	0.6	0 – 17	0.77	0	0 – 17	0.77	0.34	0 – 17
Gnathostoma procyonis	0		0 – 1	0	-	0 – 1	0.04	0.03	0 – 1
Physaloptera sp.	0.42	0.3	0 – 9	7.75	5.87	0 – 48	8.15	2.38	0 – 80
Molineus barbatus	0.06	0.06	0 – 2	0	-	-	0	-	-
Baylisascaris procyonis	0.03	0.03	0 – 1	0	-	-	0	-	-

Table 2.8: Abundance of *Stichorchis subtriquetrus* in beavers collected from Georgia (GA), South Carolina (SC), and the U.S. Department of Energy's Savannah River Site (SRS), USA, 2013-2018.

	GA (N=31)			SC (N=11)			SRS (N=20)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Stichorchis subtriquetrus	16.68	4.82	0 – 115	31.5	12.6423	0 – 103	21.2	5.56	0 – 135

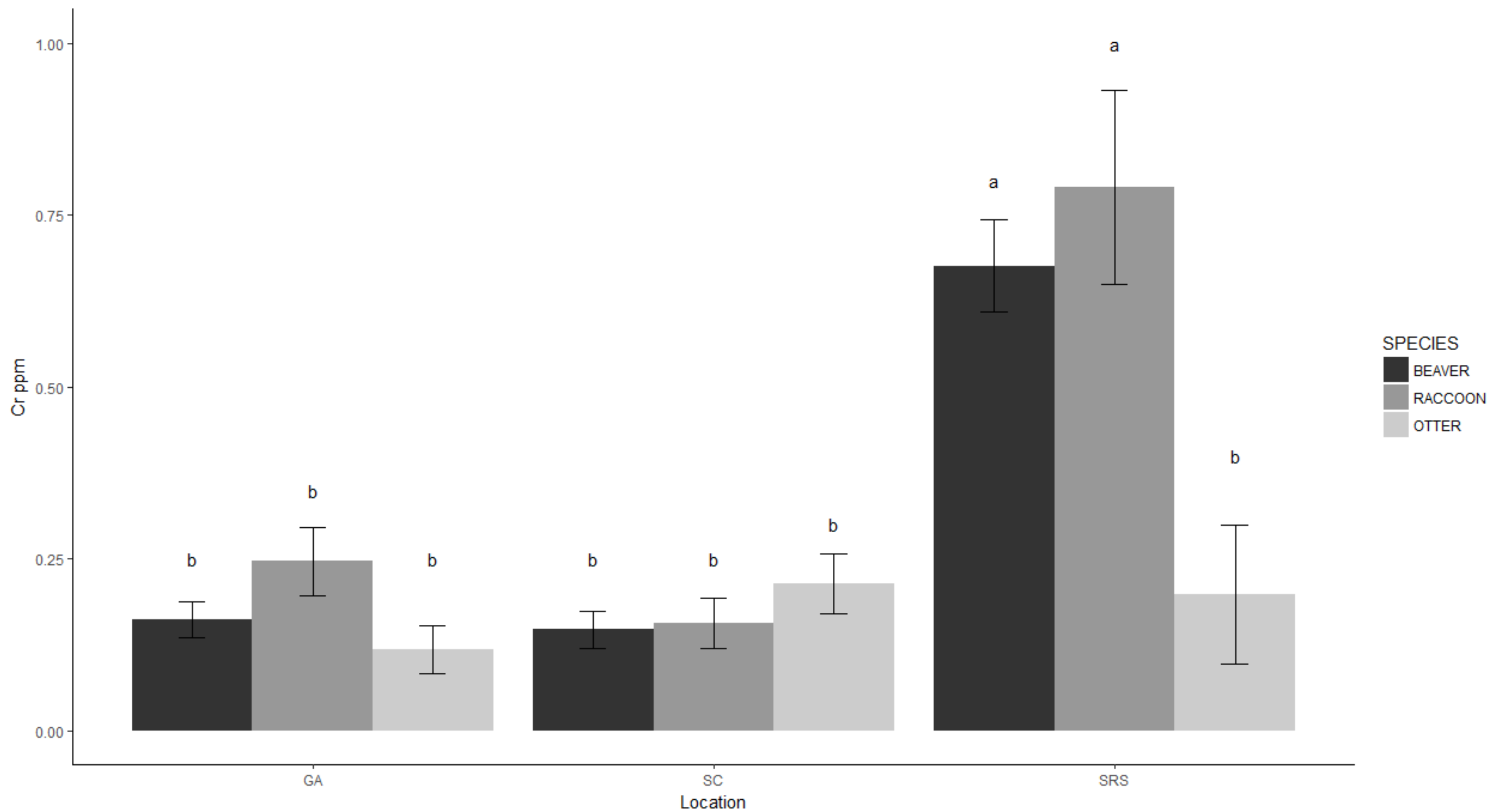

Figure 2.1: Comparison of Cr concentration (ppm; d.w.) with ±1 SE between otters, beavers, and raccoons collected from the U.S. Department of Energy's Savanah River Site (SRS) and reference sites in South Carolina (SC) and Georgia (GA). Bars sharing the same letter are not significantly different (i.e. 'ab' is not different from 'a' nor 'b').

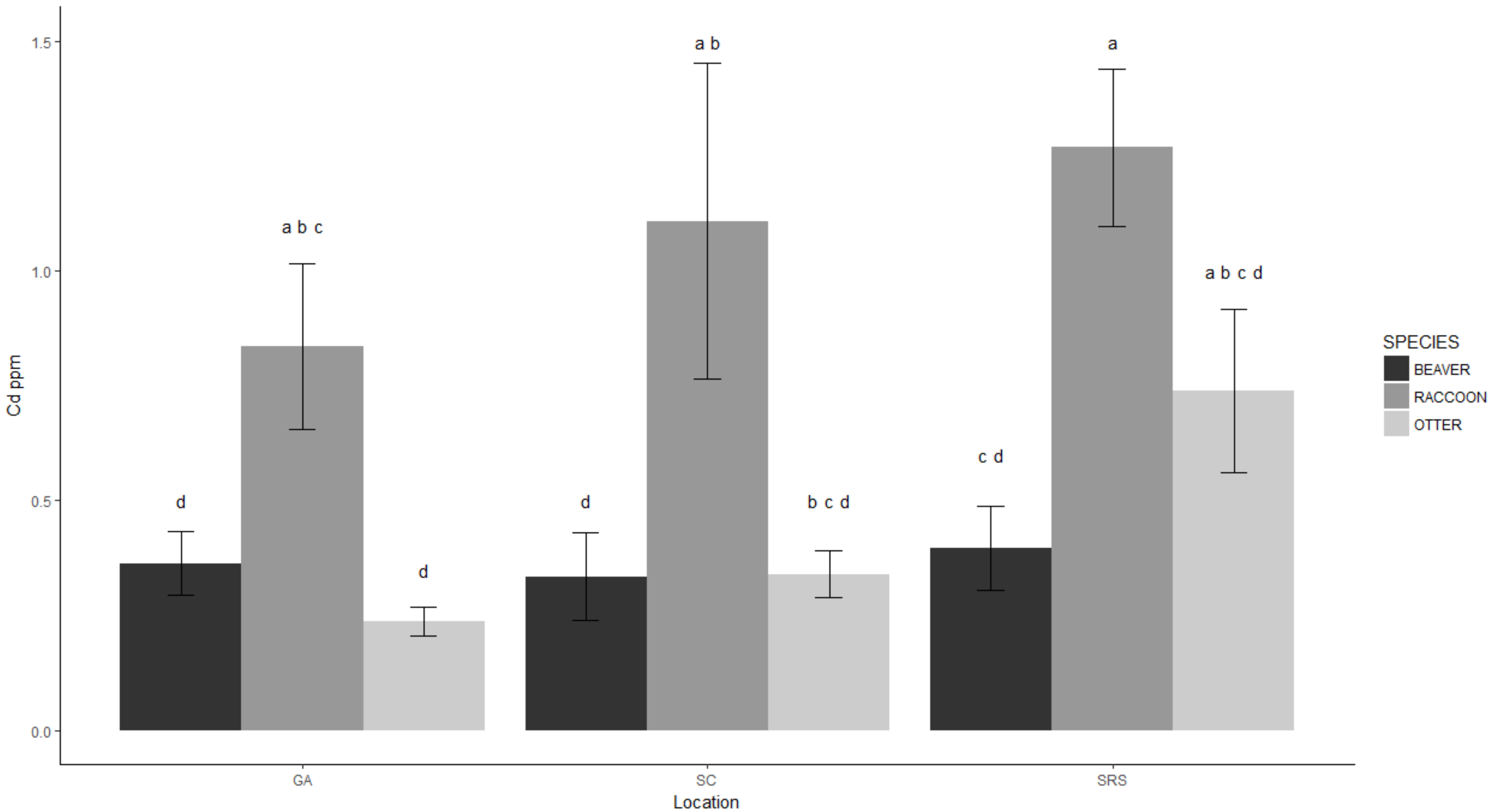

Figure 2.2: Comparison of Cd concentration (ppm; d.w.) with ±1 SE between otters, beavers, and raccoons collected from the U.S. Department of Energy's Savanah River Site (SRS) and reference sites in South Carolina (SC) and Georgia (GA). Bars sharing the same letter are not significantly different (i.e. 'ab' is not different from 'a' nor 'b').

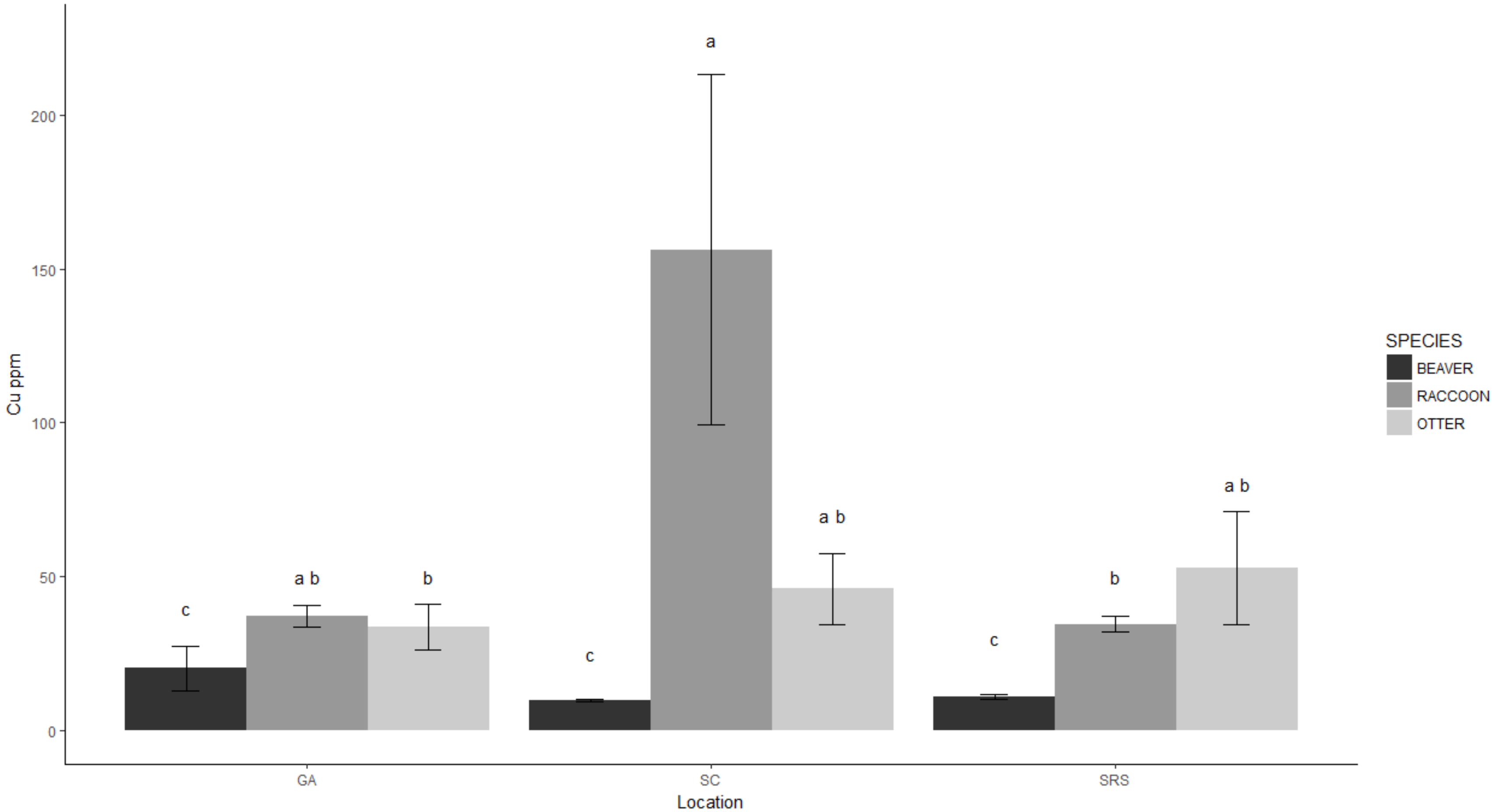

Figure 2.3: Comparison of Cu concentration (ppm; d.w.) with ±1 SE between otters, beavers, and raccoons collected from the U.S. Department of Energy's Savanah River Site (SRS) and reference sites in South Carolina (SC) and Georgia (GA). Bars sharing the same letter are not significantly different (i.e. 'ab' is not different from 'a' nor 'b').

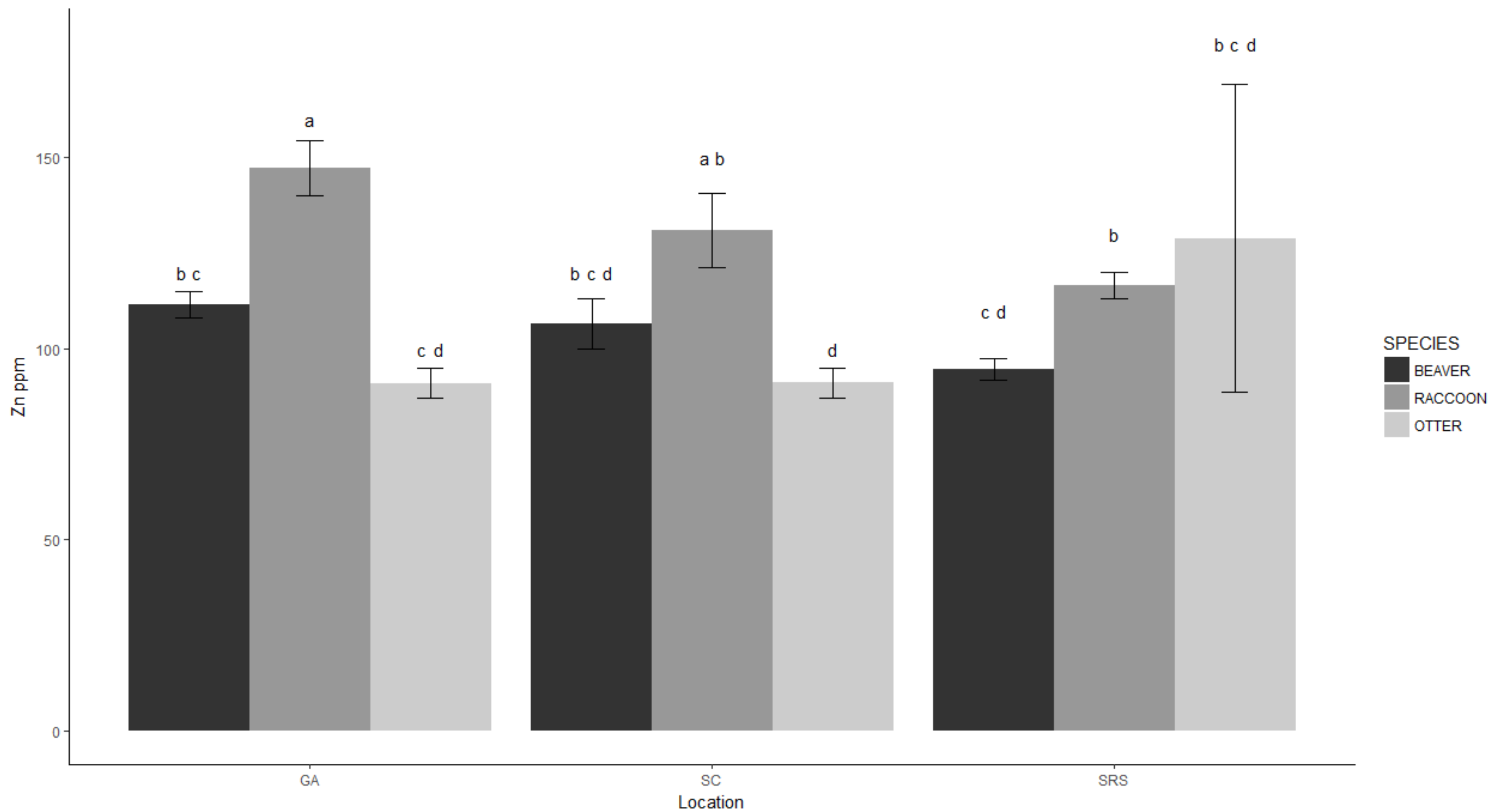

Figure 2.4: Comparison of Zn concentration (ppm; d.w.) with ±1 SE between otters, beavers, and raccoons collected from the U.S. Department of Energy's Savanah River Site (SRS) and reference sites in South Carolina (SC) and Georgia (GA). Bars sharing the same letter are not significantly different (i.e. 'ab' is not different from 'a' nor 'b').

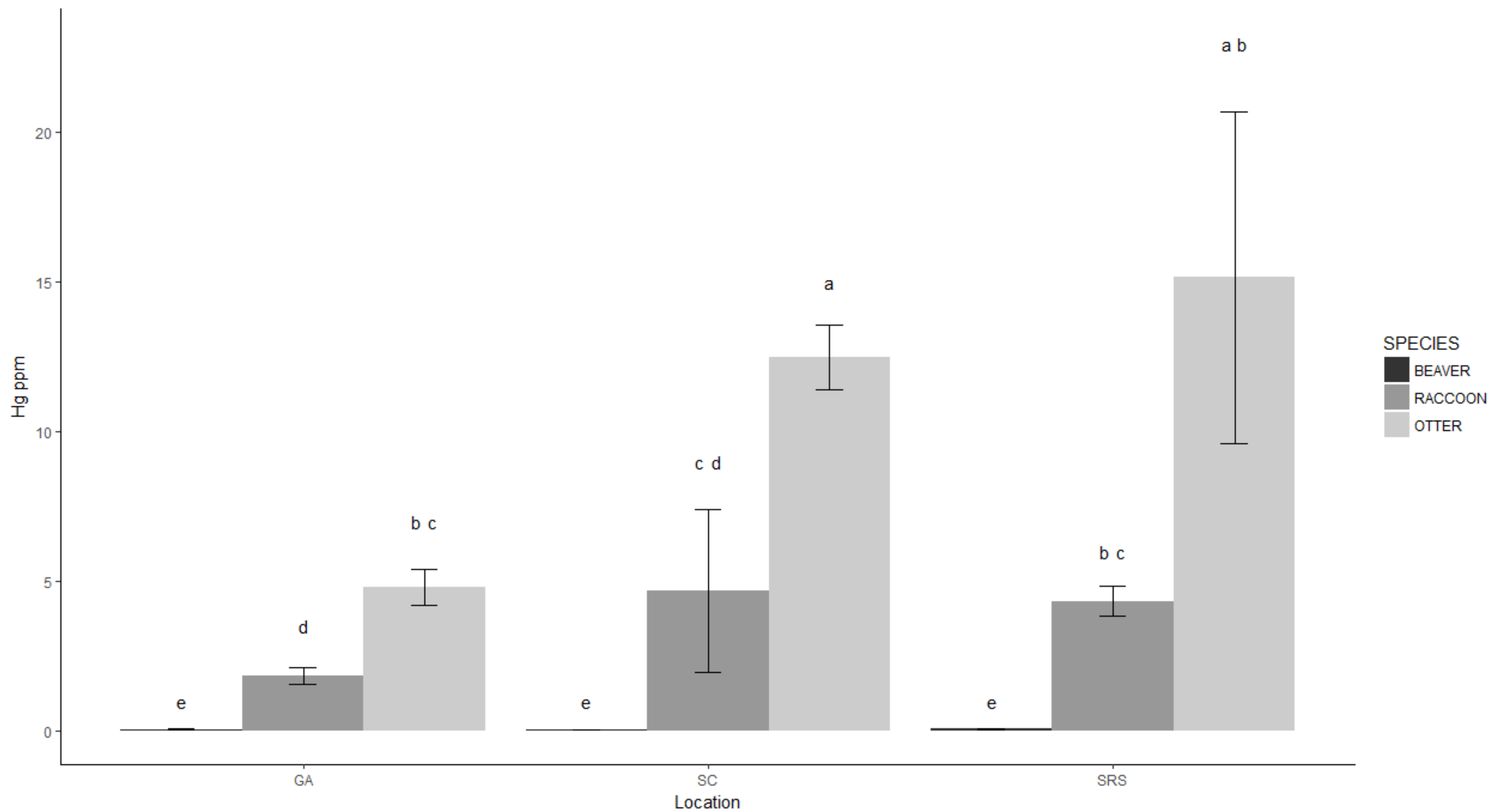

Figure 2.5: Comparison of Hg concentration (ppm; d.w.) with ±1 SE between otters, beavers, and raccoons collected from the U.S. Department of Energy's Savannah River Site (SRS) and reference sites in South Carolina (SC) and Georgia (GA). Bars sharing the same letter are not significantly different (i.e. 'ab' is not different from 'a' nor 'b').

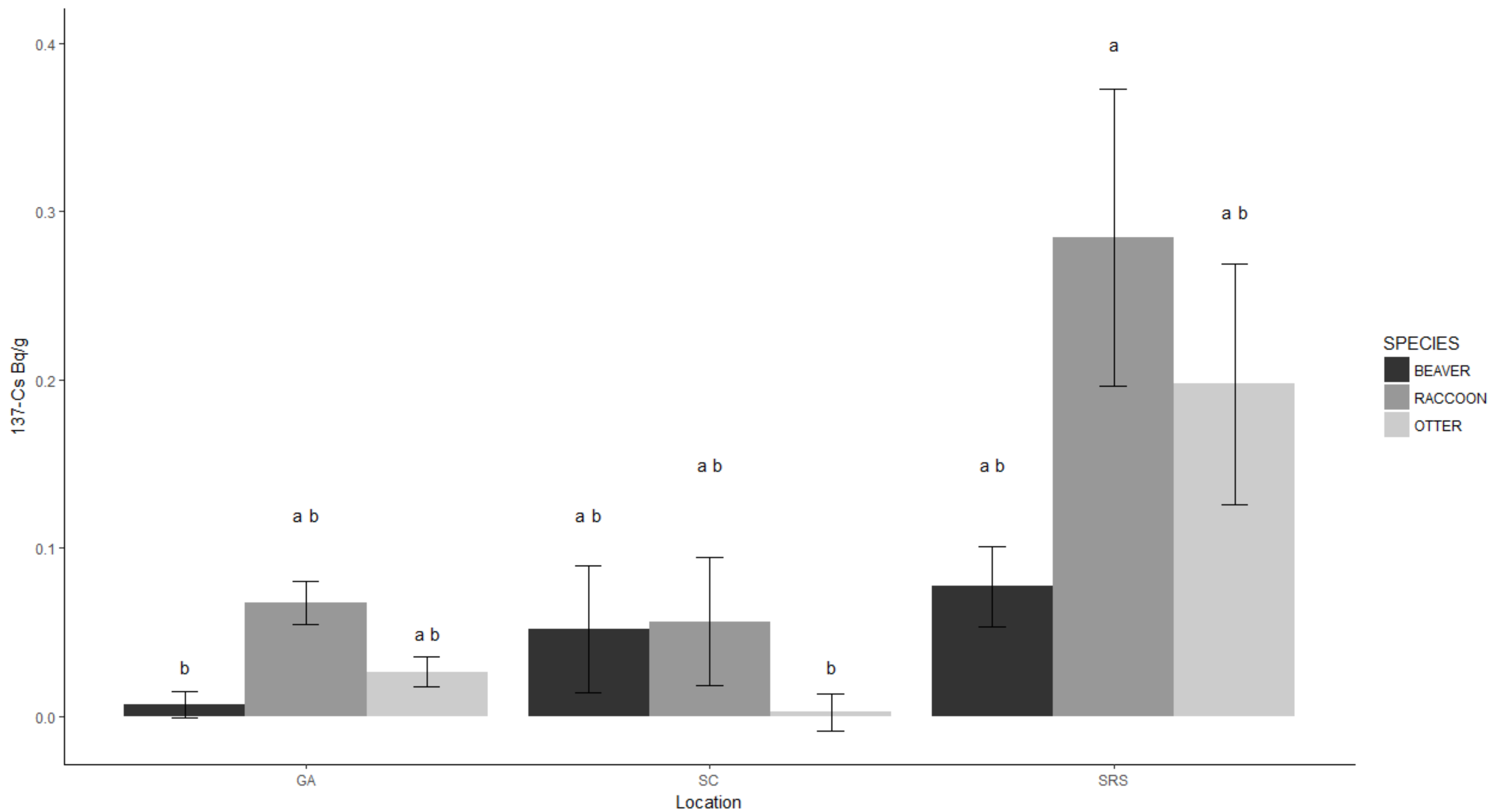

Figure 2.6: Comparison of ^{137}Cs concentration (Bq/g; d.w.) with ±1 SE between otters, beavers, and raccoons collected from the U.S. Department of Energy's Savanah River Site (SRS) and reference sites in South Carolina (SC) and Georgia (GA). Bars sharing the same letter are not significantly different (i.e. 'ab' is not different from 'a' nor 'b').

CHAPTER 3

INFLUENCE OF SPACE USE ON RADIOCESIUM UPTAKE IN RACCOONS ON THE U.S. DEPARTMENT OF ENERGY'S SAVANNAH RIVER SITE

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ABSTRACT

Contaminants are rarely distributed homogeneously within the environment and their bioavailability to organisms can vary greatly based on several biotic and abiotic factors. It has long been understood that an animal's theoretical contaminant exposure is dependent on not only its consumption rate of contaminated food items, metabolism, and bodyweight, but its space use in relation to contaminant distribution. While this has been documented in several simulation studies, there are few empirical studies directly linking animal space use to contaminant accumulation within organisms. In this study we investigate the how raccoon space use influences their contaminant concentrations in a ^{137}Cs contaminated landscape. We estimated 50% core use areas and 95% home ranges for raccoons and created models to determine how overall home range size, as well as the portion of core use areas that overlapped with contaminated areas, influence raccoon whole-body ^{137}Cs concentrations. We also constructed resource selection functions to investigate the underlying landscape variables influencing raccoon movement in a predominantly upland pine landscape with interspersed riparian and upland hardwood habitats. Mean 95% home range size and 50% core use areas for 5 male and 1 female raccoons were estimated to be 805±53ha and 166±9ha ($\bar{x} \pm 1$SE), respectively. Whole body ^{137}Cs concentrations were variable among sampled raccoons, and percent core area overlap with contaminated areas had a significant positive effect on whole-body ^{137}Cs concentrations. Raccoons selected for riparian areas, upland hardwood forest, and wetland scrub forest, habitats generally associated with good forage and den locations. These findings demonstrate the importance of considering an animal's space use when interpreting contaminant concentrations in wildlife and designing ecotoxicology studies.

Indicator species with a large home range in proportion to the contaminated area may not be representative of the area of interest. This could act as a dilution effect and make the result of the study difficult to interpret.

INTRODUCTION

Contaminants are rarely homogeneously distributed within the environment (Taylor et al 2005). Rather, extensive spatial and temporal variability in the bioavailability of contaminants exists that is modulated by a complex suite of abiotic and biotic factors. For example, the distribution and bioavailability of many contaminants can vary among habitats within an ecosystem due to variability in soil chemistry, community composition, and proximity to anthropogenic inputs (Bai et al. 2011, Brisbin et al. 1974, Pak and Bartha 1998). Thus, understanding how wildlife interact with habitats within their environment is critical for quantifying contaminant exposure and assessing ecological risk. As animals move throughout the landscape they may be exposed to contaminants by consuming contaminated soil, water or food, absorption through the skin, and inhaling particles and volatile compounds (Sample and Suter 1994). The magnitude of exposure and its effect on populations is thus directly related to the spatial distribution of contaminants, the space use of the exposed organisms, and the distribution of quality habitat (Bell et al. 1993, Purucker et al. 2007). This makes animal movement an important consideration when selecting species for ecotoxicology studies.

Use of animal movement data is becoming more popular in both aquatic and terrestrial ecotoxicology, not only to identify where animals are exposed to contaminants, but to evaluate changes in *in situ* survival due to exposure, as well as behavioral endpoints such as activity or avoidance (Ackerman et al. 2007, Hinton et al. 2015, Hellstrom et al. 2016). For example, Ackerman et al. (2007) used movement data to elucidate how differences in foraging areas, habitat use, and foraging strategy influenced mercury concentrations in American Avocets (*Recurvirostra americana*) and Black-necked Stilts (*Himantopus mexicanus*). Similarly, movement data has been useful in explaining variation in mercury concentration in little auks (*Alle alle*) due to differences in habitat use during the non-breeding season (Fort et al. 2014), how foraging ecology in northern elephant seals (*Mirounga angustirostris*) influences mercury bioaccumulation (Peterson et al. 2015), and helped identify the exposure of White-faced Ibis (*Plegadis chihi*) to persistent organic pollutants at wintering areas (Yates et al. 2010). Recently, GPS telemetry has been paired with dosimetry technology to allow researchers to directly measure radiological exposure in relation to animal movements (Hinton et al. 2015). With the growing popularity of satellite telemetry, it is becoming possible to examine how space use at fine spatio-temporal scales influences contaminant exposure. This is of particular importance when contaminants that vary at fine spatial scales are the subject of interest.

Radiocesium (^{137}Cs) is a naturally occurring radioactive isotope with a physical half-life of 30.2 years, but more commonly occurs within ecosystems from anthropogenic inputs as a product of nuclear fission (Jannik 1999, Paller et al. 2014). While ^{137}Cs can be detected globally due to fallout from nuclear power plant accidents, nuclear weapons testing, and nuclear energy production, it typically is heterogeneously distributed at local scales as activity concentrations are highly influenced by distance from the source, soil characteristics, and hydrology (Brisbin et al. 1974, Carlton et al. 1992, Bobryk et al. 2013). Radiocesium is an ideal contaminant to study how habitat use influences wildlife contaminant exposure due to its relatively short biological half-life (Fendley et al. 1977, Paller et al. 2014, Kennamer et al. 2017). For example, the biological half-life of ^{137}Cs, the time it takes for 50% of a substance to be eliminated through metabolic turnover or excretion (Jenkins et al. 1969), was found to be 5.6 days for Wood Ducks (*Aix sponsa*) and 30.1 days for green tree frogs (*Hyla cinerea*) (Dapson and Kaplan 1975, Fendley et al. 1977). The biological half-life of ^{137}Cs in the domestic pig (*Sus scrofa*) and gray fox (*Urocyon cinereoagenteus*) also is approximately 30 days (Shannon et al. 1965, Jenkins 1969). Given the short biological half-life of ^{137}Cs in vertebrates, concentrations at the time of sampling are a reflection of recent exposure and thus more sensitive to recent habitat use than other contaminants with longer biological half-lives, such as lead, which has a biological half-life in bone of approximately 16 years (Borjesson and Mattsson 1995).

The raccoon (*Procyon lotor*) has been used as an indicator species to monitor a suite of contaminants including trace elements, persistent organic pollutants, and radionuclides (Gaines et al. 2002, Porcella et al. 2004, Souza et al. 2013, Hernandez et al. 2017). Raccoons are a useful indicator species because they are widely distributed and abundant throughout most of North America (Lotze and Anderson 1979, Gaines 2002). Raccoons also are generalist omnivores that feed in both aquatic and terrestrial systems, thus having the potential to accumulate contaminants from within a variety of habitats within a landscape (Bigler et al. 1975, Herbert and Peterle 1990, Souza et al. 2013). Further, given that raccoons have a relatively small home range in many ecosystems, contaminant concentrations likely reflect exposure on a small spatial scale (Beasley et al. 2007, 2011; Gaines et al. 2000).

Previous studies have created spatially explicit models to predict levels of ^{137}Cs in a variety of wildlife including white-tailed deer (*Odocoileus virginianus*), wild pigs (*Sus scrofa*), and raccoons (*Procyon lotor*) (Gaines et al. 2005a, Gaines et al. 2005b, Bobryk et al. 2013). Gaines et al. (2005a) created a spatially explicit model that predicted raccoon occupancy throughout the Savannah River Site (SRS), and estimated ^{137}Cs burdens of raccoons inhabiting a contaminated wetland. Results of the model predicted that ^{137}Cs burdens were less than would be expected if homogenous use of the contaminated areas was assumed (Gaines et al. 2005a). However, this model was developed using VHF telemetry data and looked at resource selection at a course resolution of 10-ha. Given the fine-scale heterogeneity of ^{137}Cs that exists on the SRS, as well as other landscapes impacted by anthropogenic ^{137}Cs contamination, resource selection models developed using finer-scale spatial resolution data may be needed to better elucidate the role of raccoon movement behavior on ^{137}Cs accumulation patterns. This study seeks to take advantage of recent advancements in GPS technology to develop a model to elucidate the relationship between whole-body ^{137}Cs burdens in raccoons as a function of fine-scale space use of contaminated environments. We hypothesized that raccoon ^{137}Cs concentrations would be directly related to the amount of time spent within contaminated areas, and that raccoon activity would be concentrated within riparian and hardwood habitats, both preferred habitats of raccoons (Boring 2002, Chamberlain 2003, Beasley et al. 2011).

METHODS

Study Area

This study was conducted on the U.S. Department of Energy's SRS, located in west-central South Carolina. The SRS is a nuclear production and research facility with an approximate area of 780 km^2 (White & Gaines 2000). Operations at SRS facilities have periodically released ^{137}Cs into the environment, usually due to abnormal operating events such as cooling coil leaks, fuel failures, or faulty storage containers (Carlton et al. 1992). The areas of the SRS that received the most extensive inputs of ^{137}Cs were Lower Three Runs Creek and Fourmile Branch, which received ^{137}Cs from R and C reactors, respectively (Carlton et al. 1992). R Reactor was the first operational production reactor at the SRS and operated from 1952 until 1964 when it was placed on inactive status (Carlton et al. 1992). While active, R reactor released 8.2×10^{12} Bq into the Lower Three Runs system, where it entered via R-Canal and drained into various canals and ponds before terminating at Par Pond, which was created to provide secondary cooling water (Carlton et al.1992). C Reactor was operational from 1955 until it was shut down for extensive maintenance in 1985 (Carlton et al. 1992). During this time, reactor basin purges were discharged into Fourmile Branch and three seepage basins. The total measured release of ^{137}Cs into Fourmile Branch was 1.2×10^{12} Bq, with the final discharge occurring in 1981 (Carlton et al. 1992). With their relatively high levels of confined ^{137}Cs, the R-Canal system and Fourmile Branch make ideal locations to study how animal movements and habitat use influence contaminant (^{137}Cs) exposure.

Field and Laboratory Methods

From November 2016 – December 2017 we captured raccoons in the riparian areas surrounding the R-Canal/Pond-A complex and Fourmile Branch. We captured raccoons in box live-traps (Tomahawk No. 108, Tomahawk Live Trap Co., Tomahawk, WI) baited with whole corn and scent tablets infused with fish oil, which were strategically placed along roads and riparian areas within 100m of the contaminated waterbodies.

Upon capture, we transported raccoons to the Savannah River Ecology Laboratory where we anesthetized them with intramuscular injections of either a ketamine-xylazine mixture (20 mg/kg and 4 mg/kg, respectively) or telazol (6 mg/kg). Once anesthetized, raccoons were ear-tagged, weighed to the nearest 0.1 kg, sexed, aged (tooth-wear technique; Grau et al. 1970), and morphological measurements were taken (total length, tail length, ear length, girth, hind foot length).

While anesthetized, we whole-body counted each raccoon to determine ^{137}Cs concentration. Raccoons were isolated in a whole-body well counter, head towards the front of the chamber, with an aquarium pump blowing cool air. We used a NaI (T1) gamma detector (Bicron Model: 6H3Q/5; S/N: BJ-124R) paired to an IBM 300-GL Personal Computer (Windows 98 OS) equipped with an onboard Canberra MCA card and controlled by Canberra Genie 2000 gamma spectroscopy software (Version 1.3). A counting window of 596-728 kiloelectron-Volts (keV) centered on 662 keV was used to record total detector absorption events from the ^{137}Cs emission of 662 keV photons. The system was calibrated daily, as counting took pace, with a traceable ^{137}Cs calibration disc (New England Nuclear Gamma Reference disc Source Set; Catalogue No. NES-101S;

Cs-137 disc; 10.04 µCi on 10/2/1985) by adjusting the system amplifier gain control to center the dis-generated peak on channel 331 (661.7keV). Thirty-minute (1800s) count times were used for counting backgrounds (a 2-L bottle filled with water to approximate raccoon geometry), while 15m count times (900s) were used for counting raccoons. Background-corrected count rates (counts per second; cps) from the ILB Series of standards were used to produce mass-specific count yields which were used to produce a predictive equation of expected yields from raccoon mass. Finally, adjusted raccoon count rates and the raccoon's mass specific yield were used to determine ^{137}Cs concentration in raccoons (Bq/g).

Once counting was complete, we fitted raccoons with a GPS collar (Litetrack RF-140 or Litetrack Iridium-150; Lotek Wireless, Inc., Newmarket, ON, CA) programed to collect locations ever 4 h. After 1 month of deployment, recapture efforts began to retrieve the collar, download the data, and determine final raccoon ^{137}Cs whole-body concentrations. A matched-pairs Wilcoxon test was performed to determine if there were differences in ^{137}Cs whole-body concentrations between initial capture and recapture. Since no significant difference was found between initial and recapture raccoon ^{137}Cs concentrations (see results), we used recapture concentrations in subsequent analyses. All animal handling practices were carried out with accompanying state collection permits and in accordance with the University of Georgia Animal Care and Use Guidelines under Animal Use Protocol A2015 05-004-Y3-A5.

Statistical analysis

We used the 'adehabiatHR' package in Program R (ver3.3.3; The R Foundation for Statistical Computing) to calculate kernel density estimates (KDE) with reference bandwidth selection (h_{ref}) to determine overall home ranges (95% UD) and core use areas (50% UD) of raccoons. Home range and core use areas were estimated only for animals with ≥30 locations (Seaman et al. 1999). Radioactive contaminated areas were designated based on aerial radiological surveys conducted in 1991 and 1998 by the Betchel Nevada Remote Sensing Laboratory (Las Vegas, Nevada), as described in Proctor (1997).

We used a linear mixed effect model to investigate the relationship between raccoon whole-body concentration of ^{137}Cs and 4 space use metrics: 1) percentage of raccoon locations that occurred in the contaminated area, 2) percentage of core use area overlap with the contaminated area, 3) percentage of home range overlap with the contaminated area, and 4) the overall home range size. Prior to analysis the distribution of whole-body ^{137}Cs concentration and the space use metrics were tested for normality and collinearity. Due to collinearity between percentage of locations in the contaminated area, percent of home range overlap with contaminated area, and percent core use overlap with the contaminated area, percent home range overlap and percentage of locations in the contaminated area were removed from the model in favor of percent core use area overlap. The resulting final model included the percent core use overlap with the contaminated area, and overall home range size as fixed effects; initial capture site (Fourmile Brand or R-Canal/Pond-A) also was included as a random effect. ^{137}Cs whole-body concentrations were natural log-transformed prior to inclusion in the model. Due to the difference in magnitude between home range area and percent core use area overlap, each were scaled by subtracting their mean and dividing by 2 standard deviations, to aid with model convergence and make coefficients directly comparable (Gelman 2008).

We used a binomial approach to estimate resource selection functions (RSFs) within home ranges using distance-based landscape variables. Land cover variables of interest were determined using the SRS habitat map which was compiled from Landsat Thematic Mapper Data collected in February, April, and July 1997 with a 30m pixel size (Pinder et al. 1998). Land cover variables were reclassified similar to that of Gaines et al. (2005a) to produce the following habitat classes of interest: industrial, pine, upland hardwood, upland scrub forest, riparian, and wetland scrub forest. We combined floodplain and water/marsh in Gaines et al.'s (2005a) classification scheme as a single 'riparian' category. We used the SRS roads layer to create separate layers for unpaved and paved roads on the SRS. We created distance raster layers for habitat classes and road edges using ArcGIS 10.5 (Environmental Systems Research Institute Inc., Redlands CA), this allowed the distance from landscape variables to be extracted to our location points (used and random) and used as the independent variables in our resource selection models. To evaluate non-random resource selection, we generated 5 random locations for every known raccoon location within each individual's home range, using the 'Create Random Points' tool in ArcGIS 10.5 (Little et al. 2016). All distance-based variables were scaled prior to modeling by subtracting their mean and diving by 2 standard deviations (Gelman 2008, Hinton et al. 2016). We evaluated collinearity using Pearson correlation. We considered variables with correlation coefficients $|r| > 0.7$ to be highly correlated and retained the variable that was most biologically relevant. We evaluated variance inflation factors (VIF) of the remaining variables to further eliminate correlated variables. All variables contained a VIF ≤ 1.47, indicating a lack of collinearity among our remaining variables (O'Brien 2007). We inferred selection when used (raccoon) locations were closer to landscape variables than our random available locations. We inferred avoidance when used locations were farther from landscape variables than random locations. We developed RSFs for raccoons using binomial generalized linear mixed models using R package 'lme4' v1.1-16. In addition to landscape variables of interest, we included individual raccoons as a random effect to account for individual variation and an unequal number of locations per raccoon. We used corrected Akaike Information Criterion (AICc) to compare competing models (Burnham and Anderson 2004). We validated our RSF using k-fold cross validation (k=10; Boyce et al. 2002) and only make inference to estimates that were statistically significant ($\alpha = 0.05$).

RESULTS

We collared 20 raccoons at R-Canal/Pond-A and Fourmile Branch from November 2016 – January 2018. We collected a total of 1,206 GPS locations for a total of 6 (5 males, 1 female) raccoons, 4 from R-Canal/Pond-A and 2 from Fourmile Branch, between March 2017 and January 2018. Twelve collars failed after being deployed and 2 raccoons were not able to be recaptured. All 6 raccoons used in this study had ≥40 locations. From these individuals, we estimated the overall mean 95% home range size and 50% core use area to be 805±53ha and 166±9ha, respectively ($\bar{x} \pm$ 1SE), respectively (Figures 3.1-3.6; Table 3.1). The mean percentage of locations in the contaminated area, percent core use area overlap with contaminated areas, and percent home range overlap were 16.47±14.12%, 10.52±9.88%, and 7.05±4.13%, respectively (Table 3.1). Our matched-pairs Wilcoxon test showed no difference was found between initial and recapture whole-body ^{137}Cs concentration ($P = 1$). Whole-body ^{137}Cs concentrations averaged 0.123±0.09 Bq/g and ranged from 0.017 – 0.573 Bq/g (Table 3.1). Only raccoon R4778 appeared to have elevated whole-body ^{137}Cs concentrations (Table 3.1). The linear mixed-effects model indicated that percent core use area overlap (Intercept: $\beta = -3.13, P = 0.002$; % Core Use Area Overlap: $\beta = 2.50, P = 0.041$) were important drivers of whole-body ^{137}Cs concentration, while overall home range size was not ($\beta = -0.26, P = 0.742$).

Our only supported model describing resource selection included all 8 modeled landscape variables (Table 3.2). Raccoons selected for areas closer to riparian area ($\beta = -0.643, P < 0.01$), upland hardwood ($\beta = -0.602, P < 0.01$), wetland scrub forest ($\beta = -0.318, P < 0.01$), and paved roads ($\beta = -0.260, P = 0.011$) (Table 3.3). Raccoons selected against areas closer to pine stands ($\beta = 0.317, P < 0.01$), upland scrub forest ($\beta = 0.368, P < 0.01$), and unpaved roads ($\beta = 0.835, P < 0.01$). Our k-fold cross-validation correctly classified 86.8% of our locations as used or random based on our resource selection model.

DISCUSSION

Traditional animal contaminant exposure models have incorporated many factors including the concentration of contaminants in food items, ingestion rate, body weight, and the portion of an animal's home range that overlaps with contaminated areas (Sample and Suter 1994). The problem with these models is they assume contaminants are evenly distributed throughout the landscape or that wildlife forage randomly with respect to contamination; if contaminant levels are related to habitat quality these assumptions do not hold (Sample and Sutter 1994). While there have been several simulation studies that demonstrate the importance of home range size, habitat preference, and the spatial distribution of contaminants on animal contaminant exposure, our study is one of few to attempt to directly relate the space use patterns of individual animals to their contaminant exposure (Linkov et al. 2002, Marinussen and van der Zee 1996, Gaines et al. 2005a, Hinton et al. 2015).

To determine how raccoon space use influences contaminant exposure, we modeled whole-body ^{137}Cs concentrations using several space use metrics and found that collection site (Fourmile Branch vs R-Canal/Pond-A) and percent core use area overlap with radiation contaminated areas were significant factors influencing whole-body ^{137}Cs concentration, while overall home range size was not. This supports our hypothesis that raccoon whole-body ^{137}Cs concentrations are positively correlated with increased use of contaminated areas, and agrees well with previous simulation models (Bell et al. 1993, Purucker et al. 2007). However, we observed substantial variability in use of contaminated areas among GPS-collared raccoons, despite the fact that all individuals were caught near the edge of contaminated areas, although our sample size was limited. This is an important consideration when choosing an indicator species that is not restricted to a contaminated area.

We found the mean raccoon home range of 805±53ha to be considerably higher than previous studies using VHF telemetry (e.g., Mississippi – 244ha male, 153ha female, Chamberlain et al. 2003; Florida – 260ha male, 66ha female, Walker and Sunquist 1997; Indiana –92ha male; 58ha female Beasley et al. 2007), including a previous study on the SRS (216ha male; 155ha female; Boring 2002). Our mean home range size was inflated by the large home range of an adult male, but even after excluding this individual the mean home range size was still >450 ha. Although the large home ranges observed in our study were possibly an artifact of small sample size, the distribution and juxtaposition of habitat types in our study likely contributed to the extent of raccoon movements. The areas of the SRS where this study occur were predominantly pine forest interspersed with upland hardwood stands and riparian areas. Hardwood forests and riparian areas correspond to food, water, and dens sites, which are important to raccoon life history (Beasley et al. 2011, 2012; Byrne and Chamberlain 2011), while pine forests are typically considered low quality habitat (Kaufman 1982). Thus, the isolation of patches of high quality habitat within a matrix of lower quality habitat likely contributed to the large home range sizes we observed.

Selection for riparian areas, upland hardwood, and wetland scrub forest are likely a function of water availability, quality forage, and good den sites (Beasley et al. 2011, 2012; Byrne and Chamberlain 2011). Raccoons extensively utilize riparian areas throughout their range, and den sites associated with hardwood forest have been shown to have an important impact on raccoon population demographics (Beasley 2011, 2012). Selection for paved roads possibly reflects the role of raccoons as generalist omnivores and scavengers (DeVault et al. 2011, Olson et al. 2012, Turner et al. 2017). Paved roads on the SRS receive the most traffic and are likely to have road-kill, which represents an important food source for raccoons. While pine forests are not typically thought of as high-quality raccoon habitat (Kaufman 1982), studies have shown that raccoons utilize pine forest under silvicultural regimes that promote soft mast (Chamberlain et al. 2003).

The selection against pine stands that we observed is possibly due to forest management practices on the SRS, with less understory vegetation occurring in pine stands at our sites.

Although sample size was a limiting factor in this study, our observations are consistent with previous modeling studies, and reflect the underlying heterogeneity in the distribution of ^{137}Cs in most contaminated environments. In this study we show that ^{137}Cs whole-body concentration is directly related to animal space use, with greater use of contaminated areas leading to increased concentrations of ^{137}Cs. We also found that although all raccoons were captured adjacent to the contaminated areas, not all raccoons were using the contaminated areas similarly. This is an important consideration when selecting indicator species for ecotoxicology studies as individuals with large home ranges may not represent extensive use of the area of interest.

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Table 3.1: Whole-Body ^{137}Cs concentration (Bq/g) and space use metrics of raccoons captured near contaminated areas of the U.S. Department of Energy's Savannah River Site in Aiken, SC between March 2017 and January 2018. 'R' designates raccoons captured near R-Canal and Pond-A, while FM designates raccoons captured near the Fourmile Branch beaver pond.

ID	Sex	Initial Capture Site	Whole-Body 137Cs concentration (Bq/g)	% Locations in Radiation Areas	50% Core Use Area (ha)	Core Use Area Overlap with Radiation Area (ha)	% Core Use Area Overlap with Radiation Area	95% Home Range (ha)	Home Range Overlap with Radiation Area (ha)	% Home Range Overlap with Radiation Areas
R4757	M	R	0.017	0.00	112	0.01	0.01	543	0.12	0.02
R4778	M	R	0.573	86.83	77	46.21	59.80	405	109.92	27.13
R4953	M	FM	0.061	1.48	108	0.00	0.00	481	23.01	4.79
R4954	M	R	0.055	2.99	214	7.03	3.28	740	24.38	3.29
R4955	M	R	0.016	7.50	461	0.03	0.01	2546	161.92	6.36
R4975	F	FM	0.013	0.00	23	0.00	0.00	113	0.80	0.70
$\bar{x}$ (SE)			0.123 (0.09)	16.47 (14.12)	166 (64)	8.88 (7.55)	10.52 (9.87)	804 (358)	53.36 (27.29)	7.05 (4.13)

Table 3.2: Summary of models describing resource selection, for raccoons captured on the U.S. Department of Energy's Savannah River site from March 2017-January 2018.

Model*	K	AICc	ΔAICc	*w*
(1|ID)+IND+RIP+PIN+UHW+USF+WSF+PAV+UPR	10	6146.02	0	1
(1|ID)+IND+RIP+PIN+UHW+PAV+UPR	8	6170.06	24.03	0
(1|ID)+IND+RIP+PIN+UHW+UPR	7	6178.37	32.34	0
(1|ID)+IND+RIP+PIN+UHW+PAV	7	6280.11	134.09	0
(1|ID)+IND+RIP+PIN+UHW	5	6285.56	139.54	0
(1|ID)+RIP+UHW	4	6321.06	175.04	0
(1|ID)+RIP+PIN	4	6376.06	230.04	0
(1|ID)	2	6524.52	378.50	0

*(1|ID) = random effect of individual raccoons, IND = scaled distance to industrial areas, RIP = scaled distance to riparian, PIN = scaled distance to pine, UHD = scaled distance to upland hardwood stands, USF = scaled distance to upland scrub forest, WSF = scaled distance to wetland scrub forest, PAV = scaled distance to paved roads, UPR = scaled distance to unpaved roads.

Table 3.3: Fixed effect parameter estimates of the best mixed-effect resource selection function for raccoons on the U.S. Department of Energy's Savannah River Site in Aiken, SC, March 2017 - January 2018.

Model Variable	β	SE	*P*
Intercept	-1.617	0.118	<0.01
IND	0.013	0.104	0.899
RIP	-0.643	0.093	<0.01
PIN	0.317	0.067	<0.01
UHW	-0.602	0.078	<0.01
USF	0.368	0.081	<0.01
WSF	-0.318	0.080	<0.01
PAV	-0.260	0.102	0.011
UPR	0.835	0.073	<0.01

IND = scaled distance to industrial areas, RIP = scaled distance to riparian, PIN = scaled distance to pine, UHD = scaled distance to upland hardwood stands, USF = scaled distance to upland scrub forest, WSF = scaled distance to wetland scrub forest, PAV = scaled distance to paved roads, UPR = scaled distance to unpaved roads.

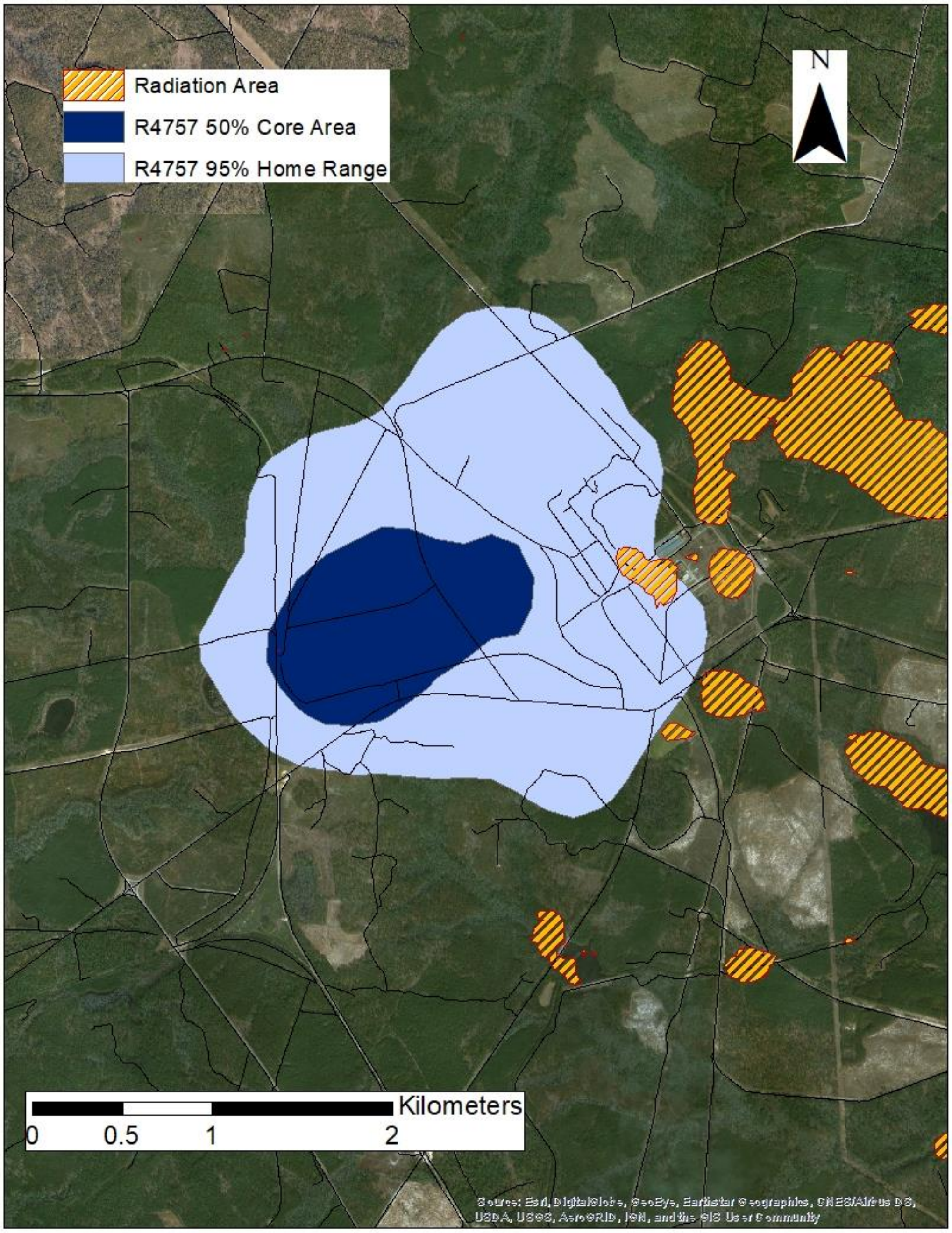

Figure 3.1: 95% home range and 50% core use area for a male raccoon, R4757, and the overlap with radiation contaminated areas, near R-Canal on the U.S. Department of Energy's Savannah River Site, Aiken, SC, USA.

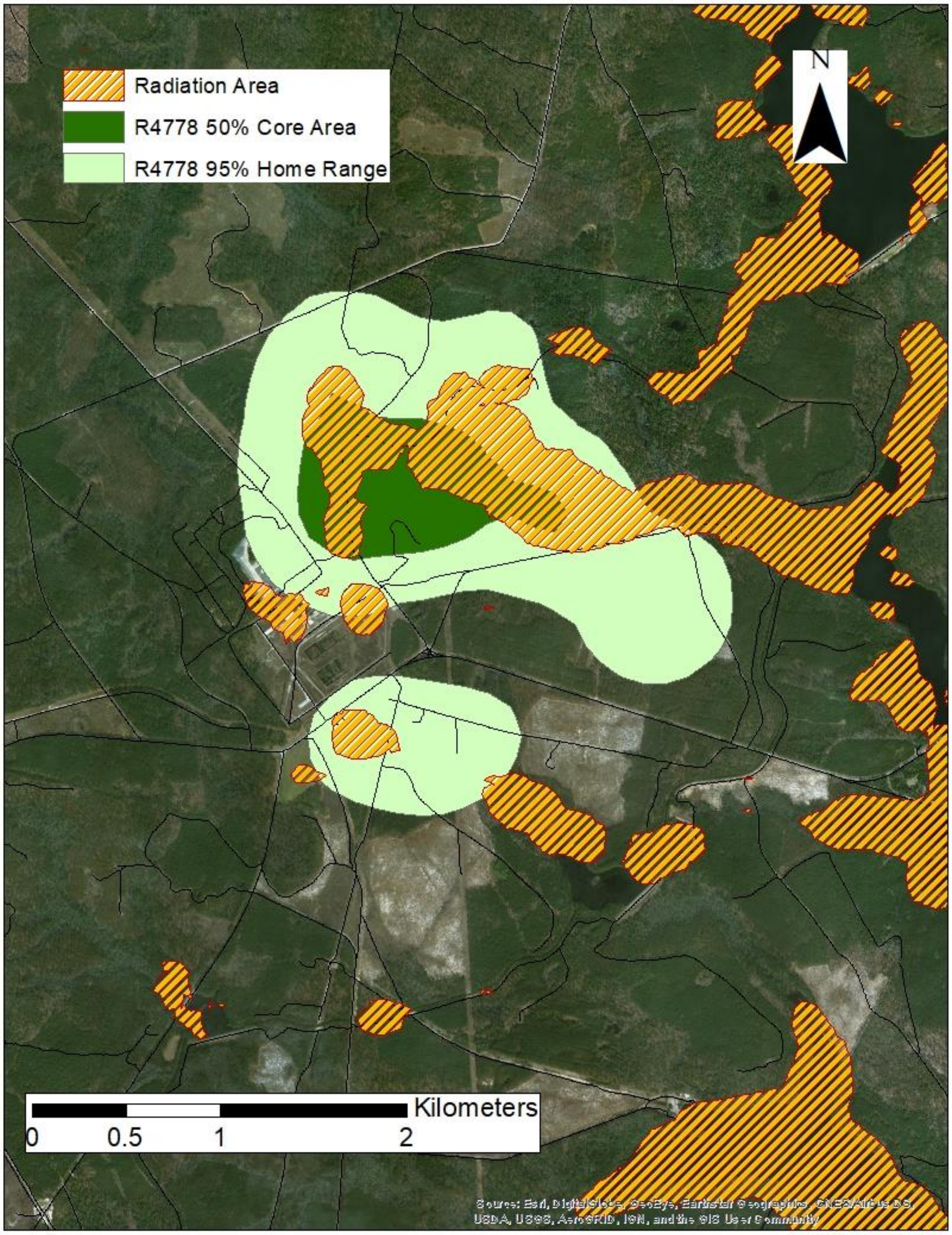

Figure 3.2: 95% home range and 50% core use area for a male raccoon, R4778, and the overlap with radiation contaminated areas, near R-Canal on the U.S. Department of Energy's Savannah River Site, Aiken, SC, USA.

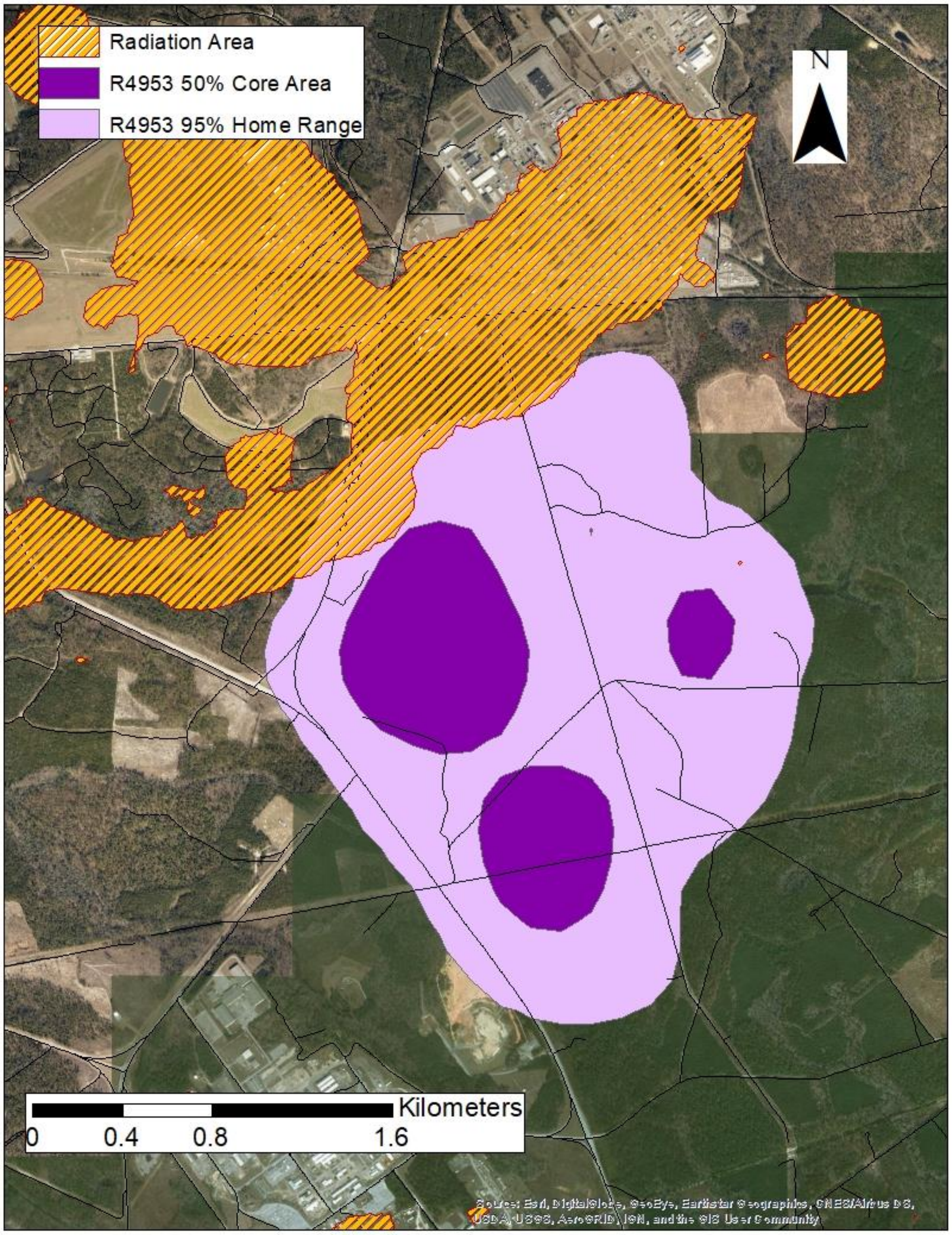

Figure 3.3: 95% home range and 50% core use area for a male raccoon, R4953, and the overlap with radiation contaminated areas, near Fourmile Branch on the U.S. Department of Energy's Savannah River Site, Aiken, SC, USA.

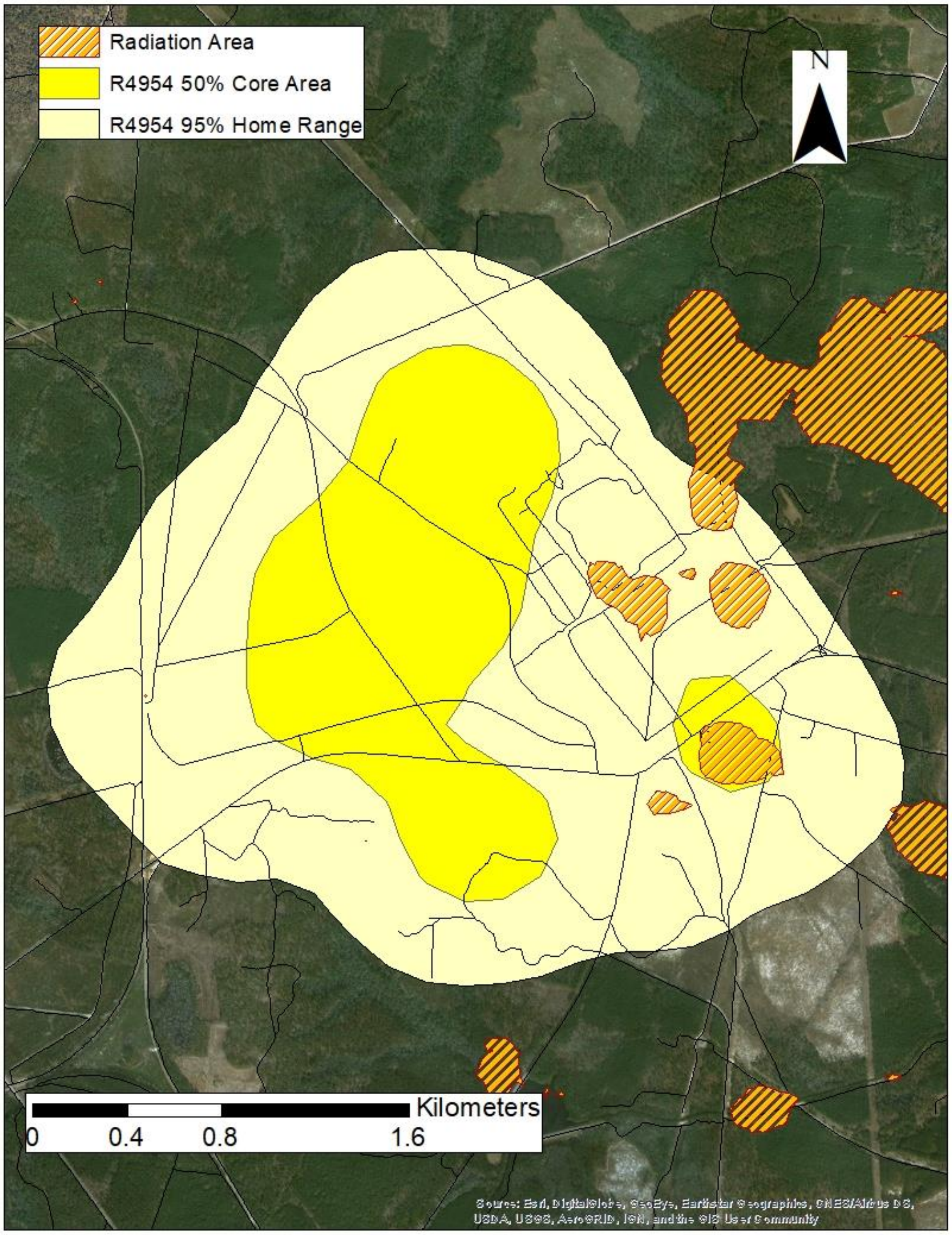

Figure 3.4: 95% home range and 50% core use area for a male raccoon, R4954, and the overlap with radiation contaminated areas, near R-Canal on the U.S. Department of Energy's Savannah River Site, Aiken, SC, USA.

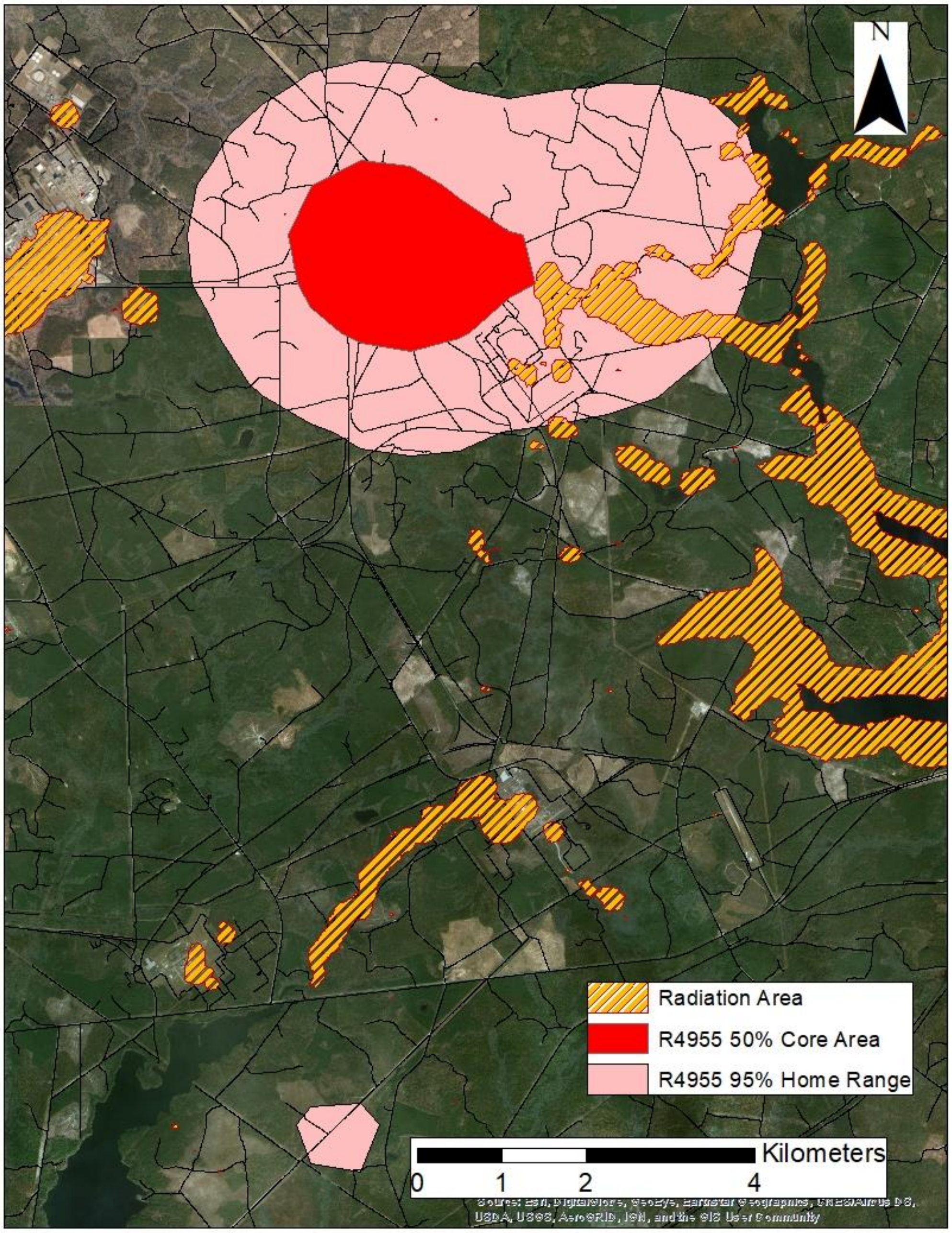

Figure 3.5: 95% home range and 50% core use area for a male raccoon, R4955, and the overlap with radiation contaminated areas, near R-Canal on the U.S. Department of Energy's Savannah River Site, Aiken, SC, USA.

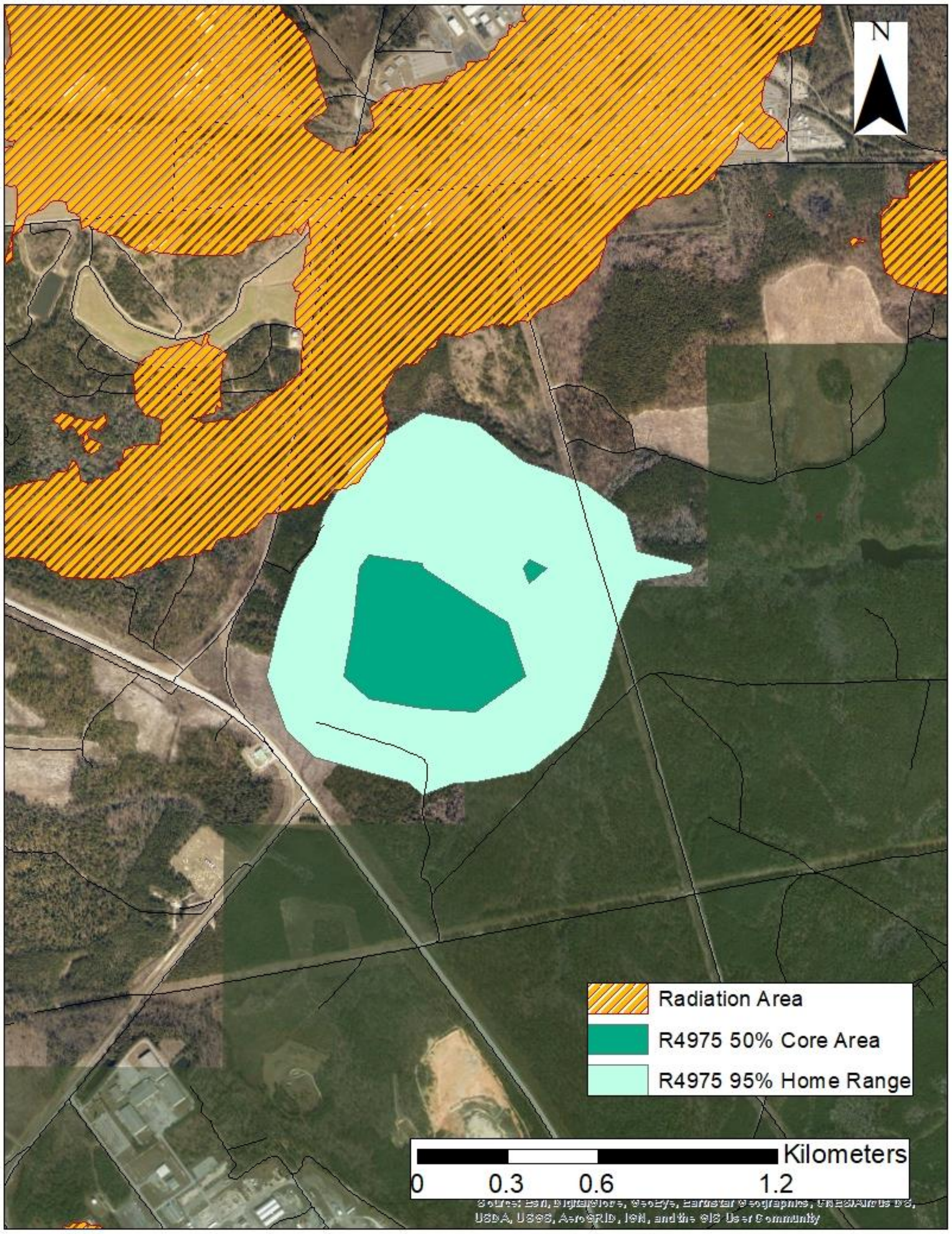

Figure 3.6: 95% home range and 50% core use area for a female raccoon, R4975, and the overlap with radiation contaminated areas, near Fourmile Branch on the U.S. Department of Energy's Savannah River Site, Aiken, SC, USA.

CHAPTER 4

CONCLUSION

Environmental contamination by trace elements (Hg, As, Cd, Cr, Cs, Cu, Ni, Pb, Se, Sr, U, Zn) and radionuclides (^{137}Cs) is a common issue throughout the world, representing a threat to the structure and function of ecosystems (Vitousek et al. 1997). To gain a better understanding of how anthropogenic stressors affect wildlife the central objectives of my thesis research were to: 1) compare trace element and radionuclide concentrations in North American river otter (*Lontra canadensis*), beaver (*Castor canadensis*), and raccoons (*Procyon lotor*) between the SRS, South Carolina, and Georgia, 2) compare contaminant concentrations in North American river otter, beaver, and raccoon as a function of trophic position, 3) determine the relationship between host contaminant concentration and parasite abundance, and diversity for each mammalian host species, and 4) develop a model capable of explaining variation in whole-body burdens of raccoons as a function of scale space use. The results of my research build on previous knowledge, and updates our understanding of contaminants in southeastern furbearers, and how space use influences contaminant uptake which can be applied to any landscape where contaminants are heterogeneously distributed.

In Chapter 2, we quantified concentrations of trace elements and ^{137}Cs in otter, beaver, and raccoons from South Carolina, Georgia, and the SRS and investigated how host contaminant concentration influences parasitism. The data reveal that Hg biomagnified as a function of trophic position, with lowest concentrations in beavers, intermediate concentrations in raccoons, and the highest concentration in river otters. We found that the highest concentrations of mercury were from otters on the SRS, while SC otters had intermediate concentrations, and GA otters had lower concentrations. This is explained by differences in soil properties as well as juxtaposition to contaminant sources (Harriss 1971) and is consistent with Cumbie and Jenkins (1974), who found that mercury concentrations in wildlife increased along a transitional gradient from the Piedmont of Georgia, through the Upper and Lower Coastal Plain, including the SRS. While the mean hepatic Hg concentration we found in otters is high in comparison to many studies of river otter in North America (Sheffy and St. Amant 1983, Wren 1986, Klenavic et al. 2008, Mayack2012, Stansley et al. 2010, Grove & Henny 2008), higher concentrations have been previously reported in parts of Georgia (Halbrook et al. 1994). The maximum hepatic Hg concentration in otters of 15.63 ppm w.w. is below the threshold of 33 ppm w.w. associated with lethality (O'Connor and Neilsen 1980). Surprisingly, muscle concentrations of ^{137}Cs did not significantly differ between the SRS and our other sampling locations, however there was a general trend of elevated ^{137}Cs among individuals sampled on the SRS.

We also show that *Stichorchis subtriquetrus* in beavers increased with hepatic Hg concentration while hepatic Hg concentration had no significant effect on the total abundance of parasites in raccoons. While previous studies have linked increased Hg concentration with the occurrence of parasites (Klenavic et al. 2008, Spalding et al. 1994) the response between Hg concentrations and immune function in wildlife requires further study (Scheuhammer et al. 2007). Species richness of parasites in raccoons increased in relation to hepatic Se concentrations. This is contrary to what has been previously reported concerning in effect of Se on parasitism (Riggs et al. 1987). To best answer how host contaminant concentrations may influence endoparasite communities, experimental infections should be conducted using hosts with known contaminant exposure.

In Chapter 3, we modeled whole-body ^{137}Cs concentrations using several space use metrics and found that the amount an animal's 50% core use area overlaps a contaminated area is an important factor in determining contaminant concentration. While there are several simulation studies that demonstrate the importance of home range, habitat preference, and the spatial distribution of contaminants on animal contaminant exposure, this was one of the few attempts to directly relate the space use patterns of individual animals to their contaminant concentrations (Linkov et al. 2002, Marinussen and van der Zee 1996, Gaines et al. 2005, Hinton et al. 2015). We also found that the home ranges of raccoons from Fourmile Branch and R-Canal/Pond-A are considerably higher than what has been previously reported in the literature using VHF telemetry (Chamberlain et al. 2003, Walker and Sunquist 1997, Beasley et al. 2007) including previous studies of raccoons from other locations on the SRS (Boring 2002). One explanation for this is the distribution and juxtaposition of habitat types within the study area. The areas of the SRS where this study occur are predominantly pine forest with upland hardwood stands and riparian areas intermixed. Hardwood forests and riparian areas correspond to food, water, and dens sites which are important to raccoon life history (Beasley et al. 2011, 2012; Byrne and Chamberlain 2011), pine forests are not typically considered high quality habitat (Kaufman 1982). This explanation is supported by our resource selection function that shows that raccoons selected for upland hardwood forests and riparian areas while selecting against pine forests.

The data presented this thesis builds upon our knowledge of anthropogenic contamination in furbearers of the southeastern United States and the potential effects it has on their parasite communities. One important finding is that animals occupying the coastal plain in the southeastern United States appear the have higher mercury concentrations than other physiographic regions. While we didn't find hepatic Hg concentrations at levels that are normally thought of as a concern for the species in question, it should be noted that wildlife populations in the Lower Coastal Plain may be at a higher risk of Hg toxicosis compared to other parts of North America due to the geology of the region. The relationship we show between space use and contaminant concentrations should be taken into consideration when choosing indicator species for ecotoxicology studies as animals with large home ranges in proportion to the contaminated area may not represent extensive use of the areas of interest.

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