ENVIRONMENTAL FATE OF RADIOCESIUM IN AQUATIC AND SEMI-AQUATIC BIOTA ON THE U.S. DEPARTMENT OF ENERGY'S SAVANNAH RIVER SITE

by

JAMES CHRISTOPHER LEAPHART

(Under the Direction of James C. Beasley)

ABSTRACT

Although studies have examined the fate of radiocesium (^{137}Cs) in biota due to anthropogenic contamination in ecosystems around the world, much of the current literature is limited to select trophic linkages and species. I conducted a comprehensive survey of aquatic and semi-aquatic organisms, including invertebrates, fish, amphibians, reptiles, and mammals, from a ^{137}Cs contaminated system on the Department of Energy's Savannah River Site to quantify ^{137}Cs activity within their body tissues. Using these data, I assessed the potential for ^{137}Cs biomagnification, the influence of ontogenetic shifts in ^{137}Cs activity between life stages of herpetofauna, and bioaccumulation rates of ^{137}Cs in amphibian larvae. Collectively, ^{137}Cs concentrations and stable isotopes were found to be highly variable among our different collective species, suggesting that biomagnification is not ubiquitous, but is instead system dependent and influenced by a variety of biotic and abiotic factors that impact contaminant uptake in exposed organisms.

INDEX WORDS: Radiocesium, Biomagnification, Bioaccumulation, Ontogenetic Shift, Food Webs, Anthropogenic Pollution, Savannah River Site

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Nuclear accidents, such as those that occurred in Chernobyl and Fukushima, serve as looming reminders that anthropogenic activities can have severe consequences through the dispersal of radionuclide contaminants (e.g., ^{131}I, ^{137}Cs, ^{90}Sr, ^{239}Pu, ^{240}Pu) into the environment (UNSCEAR 2008; Kawamura et al. 2011; Steinhauser et al. 2014). These accidents not only released radionuclides into the landscape immediately surrounding the reactors, but also dispersed contaminants into the atmosphere, exposing fish, wildlife, and human populations hundreds or more kilometers from the accident sites (UNSCEAR 2008; Kawamura et al. 2011; Steinhauser et al. 2014). However, nuclear contamination is not limited to these iconic accidental worst case scenarios, as lesser emissions and nuclear weapons testing have deposited radionuclides worldwide, making radiocontamination a global issue (Prăvălie 2014). Furthermore, use of nuclear technologies for the production of energy is a growing industry, with 446 nuclear reactors currently active, spanning 30 countries worldwide, and additional reactors under construction (IAEA 2017). Given the widespread distribution of anthropogenic radionuclides currently in the environment, and the potential for further contamination, there is a direct need to better understand the transport and ecological fate of radionuclides within ecosystems.

Radiocesium (specifically ^{137}Cs) is a radionuclide of particular concern not only because of its relatively long physical half-life of 30.2 years, but also because its chemical properties are similar to potassium (K), allowing it to accumulate in the soft tissues (particularly skeletal muscle) of exposed biota, especially in environments deficient in potassium (K) (Paller et al. 2014). Previous studies have demonstrated that ^{137}Cs biomagnifies in fish communities within contaminated systems (Carlsson and Lidén 1978; Rowan et al. 1998; Sundbom et al. 2003, Zhao et al. 2001). For example, Rowan et al. (1998) demonstrated that fish had a two to four-fold increase in tissue concentrations of ^{137}Cs as they increased in trophic position. Carlsson and Lidén (1978) also found that pike (*Esox lucius*) contained higher concentrations of ^{137}Cs (two-fold increase) compared to roach (*Rutilus rutilus*) and bream (*Abramis brama*), fish of lower trophic levels. Similar to fish within aquatic systems, studies focusing on terrestrial wildlife have also demonstrated that certain predators also exhibit higher ^{137}Cs concentrations than their prey (Pendleton et al. 1964; Hanson et al. 1967; Lowe and Horrill 1991). For example, mountain lions (*Puma concolor*) have been found to accumulate higher levels of ^{137}Cs than mule deer (*Odocoileus hemionus*), their primary prey (Pendleton et al. 1964). This increase in contaminant burden between predator and prey suggests ^{137}Cs likely biomagnifies in both terrestrial and aquatic food webs.

Despite the many studies that demonstrate ^{137}Cs has the potential to biomagnify within a specific taxonomic group (Pendleton et al. 1964; Hanson et al. 1967; Carlsson and Lidén 1978; Lowe and Horrill 1991; Rowan et al. 1998; Sundbom et al. 2003, Zhao et al. 2001), others have suggested that this may not necessarily be the case when multiple food web links, some consisting of multiple taxonomic groups, are considered. For example, detritorivorous invertebrates collected from forests contaminated by the Fukushima Dai-ichi Nuclear Power Plant accident had elevated levels of ^{137}Cs compared to carnivorous species (Ishii et al. 2017), findings similar to that of other studies examining ^{137}Cs in invertebrate communities (Rudge et al. 1993; Sakai et al. 2016). Furthermore, studies examining ^{137}Cs concentrations in aquatic biota in the SRS's Pond B and Par Pond reservoirs have failed to provide widespread evidence in support of ^{137}Cs biomagnification in American alligators (*Alligator mississippiensis*; Brisbin 1989, Brisbin et al. 1997). Whole body ^{137}Cs concentrations in hatchling alligators captured from Pond B were found to have an average of 0.49 Bq/g live weight (Brisbin 1989), compared to 6.5-7.0 Bq/g (wet weight) in largemouth bass from the same system (Whicker et al. 1989). Even when fed a diet of Pond B largemouth bass, Brisbin et al. (1997) discovered that ^{137}Cs concentrations in captive adult alligators were approximately 0.42 Bq/g live weight, suggesting ^{137}Cs does not biomagnify in alligators within the Pond B system when they consume only largemouth bass. These data suggest factors other than diet and trophic position can play an important role in the potential for biomagnification in contaminated ecosystems. Therefore, given the disparate evidence for and against biomagnification of ^{137}Cs, there is a need for a more holistic approach to elucidate contamination across a large gradient of biota within a single system. In particular, data that elucidate ^{137}Cs concentrations of all biota within the entire food web of the contaminated ecosystem are needed to gain a more comprehensive understanding of the environmental fate of ^{137}Cs and the underlying factors contributing to its biomagnification within food webs.

In addition to diet and foraging locality, differences in life stages and associated variation in size, age, and metabolism also could affect ^{137}Cs concentrations in exposed individuals (Carlsson and Lidén 1978; Peters et al. 1996; Rowan et al. 1998; Sundbom et al. 2003, Zhao et al. 2001). For some species, differences in contaminant concentrations between juveniles and adults within the same species can be attributed to ontogenetic shifts in foraging strategy or diet as the organism ages and increases in size (e.g., Carlsson and Lidén 1978). For example, ^{137}Cs concentrations in perch (*Perca fluviatilis*) have been shown to increase as individuals age and shift their diet from plankton to fish (Carlsson and Lidén 1978). Similarly, pike have increased ^{137}Cs activity as they increase in body size, likely due to an increase in prey uptake (Carlsson and Lidén 1978). Rowan et al. (1998) also observed differences in ^{137}Cs levels between immature and mature fish, with adults having elevated levels of ^{137}Cs compared to juveniles, a trend partially explained by changes in diet and associated differences in metabolism as these organisms mature. Given that ontogenetic shifts in foraging strategies and diet can influence ^{137}Cs concentrations in fish, it is plausible similar patterns of contaminant uptake may occur in other taxonomic groups, although this has largely been unexplored.

Within freshwater ecosystems, there are numerous species of herpetofauna that span multiple trophic levels during their lifespan due to differences in foraging strategy and diet between juvenile and adult life stages. Thus, many reptiles and amphibians are optimal model organisms for assessing the effects of ontogenetic shift in foraging strategy and diet on tissue concentrations of ^{137}Cs. Indeed, ^{137}Cs activity in yellow-bellied sliders (*Trachemys scripta*) have been shown to increase with body mass (Peters and Brisbin 1996), partially due to an ontogenetic shift in diet from invertebrates to plants as the turtle increases in size (Hart 1983), or due to potential differences in dietary potassium intake (Peters and Brisbin 1996). Other species of herpetofauna, such as cottonmouths (*Agkistrodon piscivorus*) also exhibit shifts in diet between juvenile and adult life stages, where juveniles feed primarily on smaller salamander and frog species (Eskew et al. 2009), while adults consume a more generalist diet that includes prey within terrestrial habitats adjacent to the aquatic system (Allen and Swindell 1948, Eskew et al. 2009). Similar to cottonmouths, bullfrogs (*Lithobates catesbeianus*) also undergo a dramatic ontogenetic shift in diet and foraging strategy as they transition between pre- and post-metamorphic developmental stages. Confined to the aquatic habitat, bullfrog tadpoles consume decaying plant matter, algae, and incidental sand/sediments (Jenssen 1967), while post-metamorphic bullfrogs forage in both aquatic and adjacent terrestrial habitats consuming invertebrate and vertebrate prey (McKamie and Heidt 1974; Werner et al. 1995). The observed ontogenetic shift in diet and foraging strategy between juvenile and adult cottonmouths and bullfrogs make these species ideal model organisms for studying differences in ^{137}Cs concentrations between life stages.

In addition to examining the influence of trophic position and ontogenetic shift on ^{137}Cs concentrations in aquatic and semi-aquatic species, understanding the rate at which ^{137}Cs accumulates within animal tissues is also of great importance when trying to understand the environmental fate of ^{137}Cs, especially in prey species. Numerous studies have examined ^{137}Cs accumulation in several species of waterfowl (Fendley et al. 1977; George et al. 1991; Kennamer et al. 2017) and in turtles (Peters and Brisbin 1996), yet other species exposed to ^{137}Cs in aquatic ecosystems are often overlooked, despite their importance in food web dynamics. Representing both ends of the trophic spectrum depending on life stage (Whiles et al. 2006), amphibians are often considered keystone species in many ecosystems (Holomuzki et al. 1994; Wissinger et al. 1999). A single wetland can produce vast numbers of metamorphic amphibians within a single year’s breeding season (Gibbons et al. 2006), thus raising concern for contaminant exposure in large populations of amphibians, some of which are often preyed on by predators that are not confined to the contaminated system. Studies investigating contaminant accumulation in amphibians have demonstrated the potential for individuals to accumulate high levels of radionuclides (Matsusshima et al. 2015; Takahara et al. 2015), but what is unknown is the rate at which amphibians accumulate these contaminants, especially radiocontaminants like ^{137}Cs. Acquiring a better understanding of these accumulation rates of ^{137}Cs in amphibians, such as bullfrog tadpoles, could provide imperative information on radionuclide accumulation in exposed amphibian populations that can be transferred to higher trophic levels and provide linkages between aquatic and terrestrial ecosystems.

My thesis research investigating the fate of ^{137}Cs will address these research gaps by providing a more holistic approach to elucidate how ^{137}Cs moves through food webs in contaminated ecosystems, and how the life history of an exposed organism can influence ^{137}Cs accumulation. This research was conducted at the United States Department of Energy's Savannah River Site (SRS) in South Carolina, USA, which represents an ideal location to conduct this research because of its historic environmental contamination with radionuclides (Cummins et al. 1991; Jannik 1999; White and Gaines 2000). Numerous radionuclides were released into aquatic systems on the SRS, particularly through natural streams, canals, and reservoirs during efforts to cool effluent from once operating nuclear reactors (Cummins et al. 1991; White and Gaines 2000). Although multiple radionuclides have been deposited into the environment on the SRS, ^{137}Cs is by-far the most notable given its propensity to accumulate in exposed biota and persist in the environment (Jannik 1999). Because of this, ^{137}Cs has been, and still is, a major radionuclide of interest on the SRS and has been extensively studied in the sediments, plants, and wildlife since the decommission of SRS's five nuclear reactors (Brisbin et al. 1974; Staton et al. 1974; Brisbin 1989; Brisbin et al. 1997; Abraham et al. 2000; Pinder et al. 2006; Paller et al. 2014; Kennamer et al. 2017).

In the present chapter, I have provided a literature review showcasing some of the past literature investigating the fate of ^{137}Cs in biota, and have suggested further considerations that I believe should be considered. In Chapter 2, I present a study to elucidate the potential for ^{137}Cs to biomagnify within aquatic food webs by quantifying ^{137}Cs concentrations in numerous wildlife species spanning multiple taxonomic groups from a contaminated system on the SRS. This research builds on the current literature demonstrating the potential for ^{137}Cs to biomagnify with increasing trophic position in an environment where the radionuclide contamination is mainly restricted to the aquatic system. In chapter 3, I focus specifically on anuran and snake species studied in Chapter 2, and examine how ^{137}Cs concentrations can differ between juvenile and adult life stages. These differences are, in part, due to ontogenetic shifts in foraging strategy and diet as the organism matures, and demonstrate how ^{137}Cs concentrations can be highly variable within a single species depending on the life stage of exposed individuals. In my final data chapter, Chapter 4, I focus specifically on bullfrogs and examine bioaccumulation of exposed tadpoles over time. By deploying large enclosures into a contaminated system and collecting stocked tadpoles at know time intervals, I was able to model ^{137}Cs uptake in bullfrog tadpoles to better understand the process of ^{137}Cs bioaccumulation in amphibians exposed within contaminated ecosystems. Collectively, this thesis research provides answers to a few of the many important, unanswered questions within the field of radioecology, and will provide this field with critical information pertaining to the fate of ^{137}Cs in aquatic ecosystems, and their adjacent terrestrial systems.

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CHAPTER 2

ENVIRONMENTAL FATE OF RADIOCESIUM ASSOCIATED WITH TROPHIC INCREASE IN A CONTAMINATED ECOSYSTEM ON THE U.S. DEPARTMENT OF ENERGY'S SAVANNAH RIVER SITE

Leaphart, J.C., A.L. Bryan Jr., and J.C. Beasley. To be submitted to Environmental Pollution.

ABSTRACT

Although biomagnification of radiocesium (^{137}Cs) has been reported in aquatic ecosystems, previous research has been limited to select trophic linkages. Few studies have included a comprehensive survey of fauna associated with aquatic, semi-aquatic, and terrestrial habitats within a single study framework. The objective of this study is to advance our understanding of the dynamics of ^{137}Cs accumulation within food webs by quantifying ^{137}Cs concentrations across a wide range of biota found within a contaminated wetland ecosystem. Specimens representing a range of different taxa, including invertebrates, fish, amphibians, reptiles, and mammals, were collected from a contaminated canal system and associated floodplain on the Savannah River Site, and tested to quantify whole-body ^{137}Cs concentrations. Biota collected for this study included aquatic organisms that were restricted to the contaminated effluent canal, and semi-aquatic organisms able to move freely between the contaminated canal and the adjacent uncontaminated terrestrial habitat. Samples from each species were further analyzed for nitrogen stable isotopes to determine the extent to which ^{137}Cs biomagnifies within food webs. Despite extensive sampling across multiple taxa, we found that ^{137}Cs concentrations were highly variable among species, and there was no evidence of biomagnification within either aquatic or semi-aquatic species. In fact, our analyses showed a non-significant, negative relationship between ^{137}Cs concentrations and trophic level in biota restricted to the aquatic system, and a non-significant positive relationship among semi-aquatic species able to forage in adjacent, uncontaminated habitats. This suggests that ^{137}Cs biomagnification is not ubiquitous, but instead is system-dependent and influenced by a suite of biotic and abiotic factors, stressing the need for sampling a

broad suite of species and environmental attributes in the assessment of the fate of anthropogenic contaminants.

INTRODUCTION

Although certain ionizing radionuclides occur naturally from radioactive elements in soil, rocks, foods, and cosmic rays (Mehra and Bala 2014), traces of radionuclide contamination from anthropogenic sources can also be found across the globe (Prăvălie 2014; Steinhauser et al. 2014), with radiocesium (^{137}Cs) being among the most common. A product of nuclear fission with a physical half-life of 30.2 yrs, ^{137}Cs has been dispersed globally from activities such as nuclear weapons testing, nuclear power generating facilities, and faulty nuclear waste disposal storage sites (Garten et al. 2000; Prăvălie 2014,). For example, the Chernobyl nuclear disaster in 1986 alone resulted in the dissemination of approximately 11,845 Petabecquerels (PBq) of multiple radionuclides throughout much of the northern hemisphere, 74-98 PBq of which was ^{137}Cs (Anspaugh et al. 1988; UNSCEAR 2008). The accident at the Fukushima-Daiichi Nuclear Power Plant in 2011 also released ~520 PBq of radiocontamination into the environment (Steinhauser et al. 2014), with approximately 12 PBq of ^{137}Cs released into the atmosphere (Chino et al. 2011) and 3.5-27 PBq into the Pacific Ocean (Kawamura et al. 2011; Bailly du Bois et al. 2012). In addition to these large-scale ^{137}Cs releases from nuclear disasters and fallout from nuclear weapons testing, the growing dependence on nuclear energy production globally (IAEA 2017) highlights the importance of understanding the fate and transport of radionuclides within ecosystems.

Similar to other radionuclides, ^{137}Cs exposure pathways include the inhalation of radioactive particles, consumption of contaminated food, water, and/or sediment, and external exposure to radioactive beta particles and gamma rays (ATSDR 2004). In aquatic organisms, it is expected that most ^{137}Cs uptake is attributed to the ingestion of contaminated sediments within the system, or by ingestion of benthic food sources that have consumed sediments (Friday et al. 1998). A potassium (K) analog, ^{137}Cs is also able to enter food webs through absorption by plant membranes before being ingested by higher trophic level consumers (Casadesus et al. 2008). Once in the food chain, ^{137}Cs accumulates in the soft tissues of biota, and thus is able to move throughout aquatic and terrestrial food webs (Paller et al. 2014). Particularly in contaminated aquatic systems, there is evidence ^{137}Cs can biomagnify in food webs with increasing trophic position (Carlsson and Lidén 1978, Rowan et al. 1998; Zhao et al. 2001; Sundbom et al. 2003, Pan and Wang 2016). For example, Rowan et al. (1998) observed a two to four-fold increase in ^{137}Cs activity with increasing trophic level in fish communities inhabiting contaminated river and lake ecosystems in Canada. Similarly, Carlsson and Lidén (1978) found a two-fold increase in ^{137}Cs concentrations in Northern pike (*Esox lucius*) compared to their prey, roach (*Rutilus rutilus*) and bream (*Abramis brama*), in Lake Ulkesjön in Sweden.

Although conclusive evidence demonstrating biomagnification of ^{137}Cs within terrestrial food webs is lacking, studies have shown that ^{137}Cs concentrations in some carnivores exceed the levels observed within their prey. For example, ^{137}Cs concentrations in cougars (*Felix concolor*) were higher than concentrations observed in mule deer (*Odocoileus hemionus;* Pendleton et al. 1964), as were activity in wolves (*Canis lupus*) compared to caribou (*Rangifer tarandus*; Hanson 1967). Similarly, red fox (*Vulpes vulpes*) in northern England and Scotland had higher ^{137}Cs concentrations than rabbits (*Oryctolagus cuniculus*) collected from the same area (Lowe and Horrill 1991), and Eurasian lynx had higher ^{137}Cs activity than either roe deer (*Capreolus capreolus*) or reindeer (*Rangifer tarandus*) (Åhman et al. 2004).

Although these studies provide evidence that ^{137}Cs has the potential to biomagnify with food webs, other studies have failed to detect a relationship between trophic position and ^{137}Cs activity in both terrestrial and aquatic food webs. For example, in forests contaminated from the Fukushima Dai-ichi Nuclear Power Plant accident, carnivorous invertebrates were found to have lower ^{137}Cs activity compared to detritivorous invertebrates that forage at lower trophic levels on decaying plant material (Ishii et al. 2017), a finding similar to that of other studies involving invertebrate communities (Rudge et al. 1993; Sakai et al. 2016). Within the Pond B system on the Savannah River Site (SRS), gizzard shad (*Dorosoma cepedianum*) were found to have similar ^{137}Cs concentrations as black crappie (*Pomoxis nigromaculatus*) and largemouth bass (Whicker et al. 1990), despite their lower trophic level diet consisting of zooplankton, phytoplankton, insect larvae, and suspended detritus (Pierce and Wissing 1981; Drenner et al. 1982). Interestingly, hatchling alligators (*Alligator mississippiensis*) collected from the Pond B reservoir on the SRS exhibited lower ^{137}Cs levels than largemouth bass (*Micropterus salmoides*) feeding at a similar or higher trophic position within the same reservoir (Brisbin 1989, Whicker et al. 1989). However, large captive alligators fed an exclusive diet of ^{137}Cs contaminated largemouth bass from Pond B consistently had lower ^{137}Cs activity compared to the bass used as their sole prey (Brisbin et al. 1997). Collectively, these studies, along with others (e.g., Anderson et al. 1973; Straney et al. 1975, Kålås et al. 1994, Wood et al. 2009), suggest that while ^{137}Cs has the potential to biomagnify within food webs, this does not occur in all taxa and trophic links due to a complex myriad of biotic and abiotic attributes within natural environments.

Given the evidence both for and against biomagnification of ^{137}Cs within ecosystems, there is a need for a more holistic approach to examine the extent to which ^{137}Cs biomagnifies across a wider breadth of trophic links within ecosystems, as well as the underlying factors contributing to the disparity in evidence for ^{137}Cs biomagnification among ecosystems. Our study aimed to address this data gap by determining the environmental fate of ^{137}Cs across an extensive suite of taxa spanning multiple trophic levels, including aquatic species confined to a contaminated effluent canal, as well as semi-aquatic species able to forage in both the contaminated and adjacent uncontaminated terrestrial environment. We hypothesized ^{137}Cs concentrations would not consistently increase between increasing trophic positions because of the unlikelihood that ^{137}Cs will biomagnify when species spanning multiple taxonomic groups are sampled within the food web, as suggested in previous literature. Furthermore, given that movement between areas of high and low contamination can influence ^{137}Cs accumulation, we hypothesized aquatic organisms confined within the contaminated system would achieve higher ^{137}Cs levels than semi-aquatic species able to move freely between the aquatic and adjacent uncontaminated terrestrial habitat.

METHODS

Study Site

This study was conducted on the U.S. Department of Energy's SRS located near Aiken, South Carolina. The SRS spans an area of approximately 780km^2 and contains four main aquatic systems that are contaminated with ^{137}Cs: Lower Three Runs Creek, Four Mile Creek, Steel Creek, and Pen Branch (Cummins et al. 1991; Carlton et al. 1992). Of the four systems, the Lower Three Runs system received approximately 8.2 x 10^{12} Bq of ^{137}Cs from contaminated R-Reactor effluent between 1954 and 1964, where it was deposited into a canal (R-Canal), which then drained into various ponds and streams before finally reaching the designated cooling reservoir, Par Pond (Cummins et al. 1991; Carlton et al. 1992). Because ^{137}Cs contamination during this time was released directly into R-Canal, contamination is primarily restricted within the sediments of the many canals, reservoirs, and associated floodplains within the Lower Three Runs system (Carlton et al. 1992). Given the relatively high levels of ^{137}Cs in the initial stretch of R-Canal compared to other radionuclide contaminated sites on the SRS, this site is an ideal location for assessing the fate of ^{137}Cs in aquatic and semi-aquatic food webs. To determine background levels of ^{137}Cs on the SRS, we also collected biota from a nearby uncontaminated site, Old Fire Pond.

Field Methods

Sediments, periphyton, and aquatic and semi-aquatic organisms were collected from a 250-m segment of R-Canal and its adjacent floodplain, as well as Old Fire Pond, between April and September, 2015 and 2016 (Figure 2.1). Collected biota included invertebrates, fish, amphibians (larval and adult), reptiles, and mammals. All work involving animals, including capture, handling, and euthanasia, were in compliance with the University of Georgia's Institutional Animal Care and Use Committee guidelines under protocols A2015 12-017-Y2-A2 and A2015-027-Y3-A2, and under South Carolina DNR collection permits #05-2015, #F-15-16, #02-2016, and #F-16-05.

<u>Sample Collection</u>:

To quantify ^{137}Cs available within the environment, sediment samples (n = 8) were collected using 3.0 cm diameter PVC pipe. Sediments were taken as core samples to a depth of 15 cm into the canal bed within the R-Canal system, approximately 30-m apart. The entire core sample was then transferred to an 8-oz. whirl-pak where it was stored until later processing. Sediment samples were also collected from Old Fire Pond (n = 5) to later compare ^{137}Cs concentrations between radiocontaminated and reference sites. Periphyton samples (mixture of algae, cyanobacteria, heterotrophic microbes, and detritus; n = 15) were also collected from R-Canal using 30cm x 30cm polycarbonate plate suspended in the water column by a floating PVC frame. Each polycarbonate plate was removed from the frame 30 days after deployment into the canal, and placed individually into gallon-storage bags.

Given the diversity of species targeted for this study, we collected organisms (up to n = 15 for each species) using a variety of sampling techniques and methods to target specific taxonomic groups and to maximize collection success. Invertebrates, fish, reptiles and amphibians were captured using partially submerged minnow traps and hoop nets that were strategically placed throughout R-Canal and our reference location. Smaller invertebrate species were also collected via seining or dip-netting among the emergent vegetation. In addition to capture via minnow traps and hoop nets, snakes and frogs were opportunistically caught by hand or snake tongs (Gentile Giant 52 Inch Snake Tongs; Midwest Tongs, Greenwood, MO), in the case of venomous species. Raccoons (*Procyon lotor*) utilizing the contaminated system were also captured using box live traps (Tomahawk Live Trap Co., Hazelhurst, WI) placed adjacent to the canal border. All captured organisms were transported to the lab for processing and analytical testing.

Laboratory Methods

<u>Sample Processing</u>:

Upon returning to the laboratory, all sediment sample were homogenized within their whirl-pak, weighed (to the nearest g), and frozen at -20°C. Periphyton samples adhering to each polycarbonate plate were scraped off and transferred to individual 50 ml centrifuge tubes, weighed (g), and frozen at -20°C. All organisms were euthanized and individually measured to obtain weight (g) and morphometric measurements (i.e., total length, body length, fork length, snout to vent length, etc.). Aquatic invertebrates were euthanized via freezing, fish and amphibians were submerged in tricaine methanesulfonate (MS-222), and snakes were sedated with ketamine prior to blunt-force trauma to the head followed by decapitation and pithing. Raccoons were euthanized via gunshot to the head, and were used for other ecotoxicology studies after a muscle sample was extracted for this study. Afterwards, all organisms and tissues were frozen at -20°C and stored until the end of the sampling season. All samples were subsequently freeze dried and homogenized into powder using either a coffee grinder or mortar and pestle, which were cleaned with 5% nitric acid and dried between uses.

<u>Stable Isotope Analyses</u>:

To estimate relative trophic position of organisms collected from R-Canal, we analyzed a random subset (n = 4 to 5) of individuals of each species for nitrogen stable isotopes using stable isotope analysis. We chose to assess trophic position using stable nitrogen isotopes because an increase in these isotopes between different species is indicative of increasing trophic position within food webs (Post 2002). Tin capsules measuring 3.5 x 5 mm (Costech Analytical Technologies Inc., Valencia, CA, USA) were filled with 1-2 mg of lyophilized, fine-grained periphyton or biota sample using disposable micro-spatulas and sterile forceps. Once the sample was added, we removed each capsule from the balance using forceps, where it was then tightly crimped into a small packet with dimensions of < 2 mm. All samples were then analyzed at the University of Georgia Stable Isotope Ecology Laboratory (Center for Applied Isotope Studies, Athens, GA, USA) for $\delta^{15}N$ values to determine relative trophic positon. Isotopes were determined via sample combustion at 1100°C, followed by gas delivery via continuous flow using an isotope ratio mass spectrometer. Results for nitrogen isotopes were provided as $\delta^{15}N = (R_{sample} / R_{standard-1}) \times 1000$, where R is $^{15}N/^{14}N$ and air N_2 was used as a standard.

<u>^{137}Cs Analysis</u>:

Radiocesium activity was determined for all samples 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 daily using a traceable sealed source of radiocesium (SREL sealed source – 0113). Each freeze-dried, homogenized sample was packed into 3mL scintillation vials that could be tested on this machine, at weights between 0.5 and 2.0g dry weight. Because of the limited sample for smaller organisms such as snails, dragonfly naiads, and mosquitofish, I pooled multiple samples together to obtain the targeted weight for analysis. In doing so, each snail, dragonfly naiad, and mosquitofish sample consisted of 15, 20, and 5 individuals respectively. The Auto-Gamma Counter was set for a radiocesium emission center at 662 kiloelectron-Volts (keV) photons with a counting region of interest between 580-754 keV to absorb and record all possible radiocesium emissions. Tissues, sediments, and periphyton samples were analyzed for

3600 second count intervals each, with empty, background samples being placed on the counting rack at every fifth sample position. An average count yield value was determined using radiocesium standards (between 1-4g in 1g increments) prepared using chicken breast with an added 745 Bq of ^{137}Cs, and minimum detectable concentrations (MDCs) were calculated using methods described by Curie (1968).

Statistical Analysis

All statistical analyses were conducted using R statistical software (R Development Core Team). Prior to analyses, we tested all ^{137}Cs and δ^{15}N data for normality and homoscedasticity using a Shapiro-Wilks test and Bartlett's test, respectfully. To test for differences in ^{137}Cs values between our reference and treatment sites, we used a non-parametric Kruskal-Wallis test accompanied with a Dunn's multiple comparison test due to non-normally distributed residuals, even after attempted transformations (log, scaled log, square-root, logit, arcsine, etc.). Using log-transformed (log(1+ δ^{15}N)); stable isotope data, we then compared relative trophic position for both aquatic and semiaquatic biota using an analysis of variance (ANOVA), followed by Tukey's HSD tests to examine specific differences δ^{15}N values between individual species, where species was our independent variable and δ^{15}N values our dependent variable. Potential biomagnification of ^{137}Cs was then modeled using linear regression analyses on non-transformed, normally distributed and homoscedastic data (tested using Non-Constant Variance Score Test). Regression models were based on average ^{137}Cs concentrations from all individuals sampled for each species, and compared with average δ^{15}N values determined from our subset of samples analyzed for nitrogen stable isotopes. We developed separate regression models for aquatic organisms restricted within the contaminated effluent canal and semi-aquatic organisms free to move between the canal and adjacent uncontaminated terrestrial habitat within the R-Canal ecosystem.

RESULTS

We collected 16 different groups of organisms from the R-Canal system, representing biota from both aquatic and semi-aquatic species (Table 2.1). Of the 16 groups of biota collected, 11 were organisms restricted to the ^{137}Cs contaminated aquatic system: aquatic snails (Family *Planorbidae*), dragonfly naiads (Family *Libellulidae*), dytiscid beetles (Family *Dytiscidae*), water scorpions (Family *Nepidae*) red swamp crayfish (*Procambarus clarkii*), green frog tadpoles (*Lithobates clamitans*), leopard frog tadpoles (*Lithobates spenocephalus*), bullfrog tadpoles (*Lithobates catesbeianus*), mole salamander larvae (*Ambystoma talpoideum*), red-spotted newts (*Notophthalmus viridescens*) and mosquito fish (*Gambusia holbrooki*). The remaining 5 species collected were considered semi-aquatic given their ability to forage within the adjacent floodplain, and included post-metamorphic leopard frogs, green frogs, and bullfrogs, cottonmouths (*Agkistrodon piscivorus*), and raccoons. Of these species, crayfish (n = 19), leopard frog tadpoles (n = 12), dragonfly naiads (n = 12), dytiscid beetles (n = 13), mosquitofish (n = 19), and bullfrogs (n = 12) were also collected from Old Fire Pond to provide background ^{137}Cs concentrations from within our study area.

Our Kuskal-Wallis test indicated that sediment and biota samples collected from R-canal had significantly higher ^{137}Cs concentrations than our reference location (Chi Squared= 150.37, df = 13, p-value < 0.001). Our Dunn's Test further indicated that ^{137}Cs concentrations were significantly higher in R-canal for all species sampled (Figure 2.2; p-values for each group < 0.05). For our stable isotope data examining differences in δ^{15}N among species (Table 2.1), our ANOVA model revealed differences in $\delta^{15}N$ values among sampled species for both aquatic (p-value < 0.001) and semi-aquatic (p-value < 0.01) biota. Tukey's HSD tests further revealed that numerous pairwise differences in $\delta^{15}N$ values existed among sampled biota, with periphyton having the lowest and mosquitofish the highest $\delta^{15}N$ values among aquatic species, and leopard frogs the lowest and cottonmouths the highest $\delta^{15}N$ values among semi-aquatic species (Figure 2.3).

As for assessing whether there was a relationship between ^{137}Cs activity and trophic position, aquatic biota restricted to the contaminated aquatic system exhibited a weak, non-significant negative relationship between ^{137}Cs concentrations and $\delta^{15}N$ values (Figure 2.4; p-value = 0.11, $R^2 = 0.23$; $y = - 0.7064x + 7.3607$). Similarly, semi-aquatic biota showed a weak, non-significant positive relationship between ^{137}Cs concentrations and $\delta^{15}N$ values (Figure 2.4; p-value = 0.37, $R^2 = 0.27$; $y = 0.5104x – 0.9360$).

DISCUSSION

Our study is among few that have extensively surveyed a broad suite of biota representing multiple taxa within an ecosystem to elucidate the potential for ^{137}Cs to biomagnify with increasing trophic position within the food web. Measured ^{137}Cs activity within the sediments, periphyton, and biota collected from our reference site and R-canal demonstrate the magnitude of contamination that remains bioavailable within our study system more than 50 years after the initial input of contaminants, and show that ^{137}Cs concentrations are highly variable among the aquatic and semi-aquatic biota community. Furthermore, we did not observe any evidence to suggest biomagnification of ^{137}Cs with increasing trophic position is occurring within our study system. In fact, among the broad suite of biota sampled in our study, we found a negative, non-significant relationship between ^{137}Cs activity and δ^{15}N values among those species restricted to the contaminated canal. Although our results may be based off a small sample size and variation among relative trophic position and ^{137}Cs burdens among individuals within a species, these findings are comparable to those from other studies that have examined multiple trophic links within a food web (Rudge et al. 1993; Friday 1998; Murakami et al. 2014; Sakai et al. 2016; Ishii et al. 2017). Thus, although some previous studies have demonstrated that ^{137}Cs is capable of biomagnifying with increasing trophic position (Carlsson and Lidén 1978, Rowan et al. 1998; Zhao et al. 2001; Sundbom et al. 2003, Pan and Wang 2016), our findings provide further evidence that ^{137}Cs biomagnification is not ubiquitous and is instead dependent on a suite of biotic and abiotic factors within the specific system measured.

In our study, sediments were found to contain the highest concentrations of ^{137}Cs among all samples collected within R-Canal, and were significantly higher than all other samples collected, except for periphyton and leopard frog tadpoles. Because R-Canal is a relatively shallow system with heavy water disturbance during rain events, ^{137}Cs bound to disturbed sediment may have been displaced into the water column and settled on our polycarbonate plates used to collect periphyton samples. Although this is a possibility, previous studies have reported ^{137}Cs concentrations in periphyton that were comparable to or higher than concentrations in sediments or other biota within the same system (Friday 1998; Murakami et al. 2014). Furthermore, periphyton can concentrate elements suspended in the water (Friday 1998), probably due to the high surface to volume ratios in algal organisms that allows for ^{137}Cs to be accumulated and measured (Whicker et al. 1989).

Previous studies have shown that ^{137}Cs uptake in both aquatic and terrestrial biota can be heavily influenced by incidental sediment ingestion while foraging (Friday 1998, Strebl and Tataruch 2007). Thus, elevated ^{137}Cs concentrations observed across all tadpole species likely reflects a combination of a diet consisting largely of periphyton, as well as incidental ingestion of sediments while foraging (Jenssen 1967). Furthermore, a large intestinal surface area relative to body size in tadpoles (Hourdry et al. 1996) could contribute to enhanced ^{137}Cs exposure and absorption when consuming sediments, periphyton, or other food items with elevated ^{137}Cs activity levels. Future studies should attempt to quantify ^{137}Cs concentrations in the gut of larval amphibians, or other organisms that may ingest large quantities of contaminated sediments, and compare gut ^{137}Cs concentrations to levels in soft tissues and their surrounding environment.

While most studies that have observed biomagnification of ^{137}Cs in aquatic systems have examined freshwater or marine species of fish (Carlsson and Lidén 1978, Rowan et al. 1998; Zhao et al. 2001; Sundbom et al. 2003), or specific trophic links in terrestrial systems (Pendleton et al. 1964; Hanson 1967; Lowe and Horrill 1991), our study included multiple species of aquatic and semi-aquatic biota found within R-Canal, spanning across multiple trophic positions. Although we were unable to examine trends among multiple fish species due to the lack of fish biodiversity in R-Canal, we were able to quantify ^{137}Cs concentrations in numerous aquatic invertebrates and herpetofauna species common within wetland ecosystems. We found that anuran larvae, crayfish, and mosquitofish contained the highest concentrations of ^{137}Cs among aquatic organisms in our study, despite their trophic position within the system. The elevated ^{137}Cs activity in these species likely reflects their increased propensity to consume food sources and incidental sediments high in ^{137}Cs contamination, thus and increased exposure through consumption. In addition to the consumption of incidental sediment, anuran larvae consume decaying plant matter, algal biofilms, micro-invertebrates, and fungi (Jenssen 1967), all of which typically have elevated activity levels of ^{137}Cs within contaminated ecosystems (Friday 1998; Murakami et al. 2014). As with anuran larvae, it is likely that the similar diets in crayfish and mosquitofish explain their relatively high levels of ^{137}Cs compared to other organisms in R-Canal. While mosquitofish exhibit mostly a planktivorous diet, they also consume algae, detritus, and microinvertebrates (Blanco et al. 2004). Likewise, crayfish tend to forage mainly on plant material, macroinvertebrates, and detritus (Correia 2003; Alcorlo et al. 2004).

Among semi-aquatic biota able to forage in both the contaminated aquatic and adjacent floodplain, we observed an overall decrease in ^{137}Cs concentrations compared to organisms restricted to the contaminated aquatic habitat despite their differences in stable nitrogen isotope values between species. Post-metamorphic frogs, cottonmouths, and raccoons were found to have lower concentrations of ^{137}Cs compared to many of the organisms restricted to the aquatic system, likely reflecting a shift in diet to include terrestrial prey outside of the contaminated canal system. This provides further evidence that organisms able to freely move between contaminated and uncontaminated systems have lower ^{137}Cs activity (Whicker et al. 1989), and that data on residence times is critical for studies assessing contaminant concentrations in organisms able to move freely between contaminated and uncontaminated habitats (Kennamer et al. 2017). Ignoring all other semi-aquatic biota collected in this study, we did observe post-metamorphic bullfrogs trending towards higher body activity of ^{137}Cs compared to other post-metamorphic frogs within the system that they often consume. However, this trend should be further evaluated with an increased sample size before making any conclusions on ^{137}Cs dynamics in frogs at different trophic positions within contaminated ecosystems.

Although diet plays a primary role in ^{137}Cs uptake in exposed biota, other biotic and abiotic factors can greatly influence ^{137}Cs accumulation. Specifically, biotic factors such as age and size (Carlsson and Lidén 1978, Rowan et al. 1998; Sundbom et al. 2003), as well as metabolism can influence ^{137}Cs concentrations in exposed biota (Peters and Brisbin 1996), as does contaminant system, distribution, and bioavailability (Avery 1996). Our findings showing that ^{137}Cs activity is variable among species inhabiting a contaminated system, and that ^{137}Cs does not biomagnify with increasing trophic position in R-Canal, further suggesting that ^{137}Cs biomagnification is not ubiquitous, but instead is system dependent and influenced by these many biotic and abiotic factors. Because the fate of ^{137}Cs is dependent on a variety of complex interactions among organisms, their diet, and the environment where they are exposed, future studies should consider all components to the fate of ^{137}Cs contamination when making any assumptions.

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Table 2.1: Radiocesium Concentrations and Nitrogen Stable Isotope Values for Biota Collected from the contaminated effluent canal (R-Canal) on the U.S. Department of Energy Savannah River Site in Aiken, SC, USA. Samples in bold text are considered to be semi-aquatic, while all other samples are solely aquatic.

Sample*	Stable Isotope Analysis [a]		Radiocesium Concentrations	
		$\delta^{15}N$ values		^{137}Cs Bq/g dry wt
	N	$\bar{X} \pm$ SE (min/max)	*N*	$\bar{X} \pm$ SE (min/max)
SED	-----	----------	8	11.32 ± 1.47 (6.77/15.08)
PERI	4	1.44 ± 0.09 (1.26/1.63)	15	10.21 ± 0.74 (6.55/14.58)
CRAY	5	2.40 ± 0.22 (1.73/3.02)	15	4.61 ± 0.22 (3.17/5.95)
GRFGTAD	4	2.79 ± 0.44 (1.58/3.6)	6	4.48 ± 0.66 (2.25/6.16)
AQSN	5	3.35 ± 0.50 (1.67/4.42)	6	1.79 ± 0.14 (1.23/2.18)
LEOPTAD	5	3.43 ± 0.24 (3.05/4.38)	8	7.81 ± 0.63 (5.43/9.56)
DRAG	4	5.05 ± 1.05 (2.8/7.15)	10	2.65 ± 0.41 (1.35/5.78)
MOLELARV	5	5.15 ± 0.43 (4.02/6.59)	9	3.19 ± 0.33 (1.88/5.07)
BULLTAD	5	5.16 ± 0.58 (3.25/6.45)	15	6.55 ± 0.61 (2.76/10.94)
NEWT	5	5.63 ± 0.62 (3.46/6.93)	14	1.78 ± 0.14 (0.75/2.57)
DYTI	5	6.05 ± 0.38 (5.0/7.38)	15	0.33 ± 0.03 (0.13/0.52)
WASC	5	6.32 ± 0.75 (4.49/8.68)	15	0.58 ± 0.08 (0.08/1.10)
GAMB	5	8.79 ± 0.26 (8.01/9.55)	15	5.08 ± 0.13 (4.23/5.99)
LEOP	**4**	**3.52 ± 0.51 (2.13/4.39)**	**8**	**0.71 ± 0.28 (0.09/2.24)**
GRFG	**5**	**3.82 ± 0.60 (2.17/5.55)**	**15**	**0.62 ± 0.10 (0.18/1.38)**
RACC	**4**	**5.55 ± 0.29 (4.85/6.16)**	**4**	**1.87 ± 0.066 (0.46/3.24)**
BULL	**5**	**5.61 ± 0.26 (4.78/6.29)**	**15**	**3.68 ± 0.40 (0.53/6.89)**
COTT	**5**	**6.48 ± 0.32 (5.92/7.61)**	**15**	**1.19 ± 0.18 (0.19/3.00)**

* *SED* sediment, *PERI* periphyton, *CRAY* crayfish (*Procambarus clarkia*), *GRFGTAD* green frog tadpole (*Lithobates clamitans*), *AQSN* aquatic snails (Family *Planorbidae*), *LEOPTAD* leopard frog tadpole (*Lithobates spenocephalus*), *DRAG* dragonfly naiads (Family *Libellulidae*), *MOLELARV* mole salamander larvae (*Ambystoma talpoideum*), *BULLTAD* bullfrog tadpole (*Lithobates catesbeianus*), *NEWT* red spotted newt (*Notophthalmus viridescens*), *DYTI* dytiscid beetles (Family *Dytiscidae*), *WASC* water scorpion (Family *Nepidae*), *GAMB* mosquitofish (*Gambusia holbrooki*), *LEOP* leopard frog, *GRFG* green frog, *RACC* raccoon (*Procyon lotor*), *BULL* bullfrog, *COTT* cottonmouth (Agkistrodon piscivorus),

[a] Subset of four to five samples were tested from each species group.

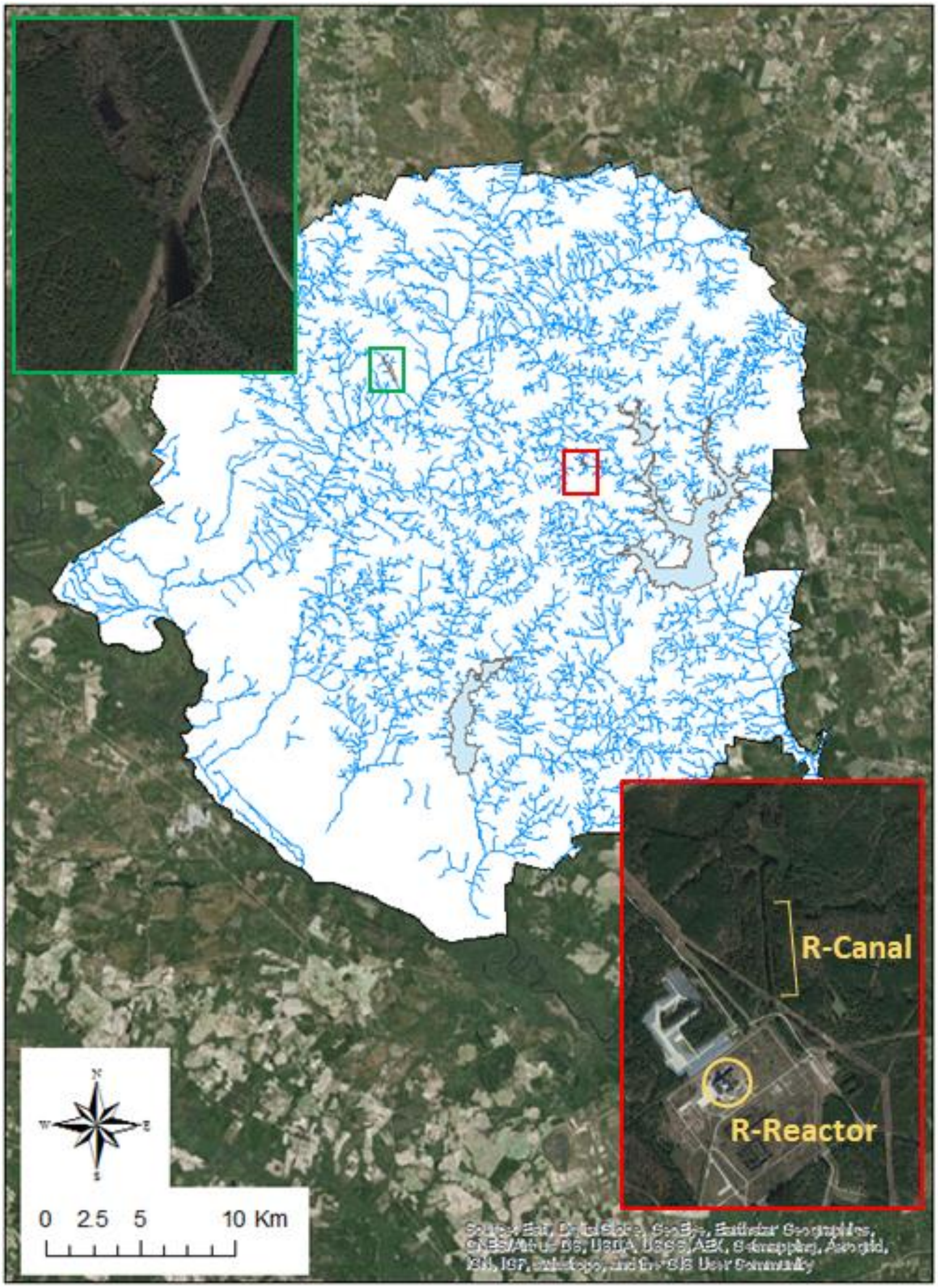

Figure 2.1: Map of the Savannah River Site (SRS) in Aiken, South Carolina, USA, with ^{137}Cs contaminated and reference site. Blue lines and shapes indicate streams and reservoirs, respectively, the green box denotes the uncontaminated reference site, Old Fire Pond, and the red box denotes the ^{137}Cs contaminated site, R-Canal.

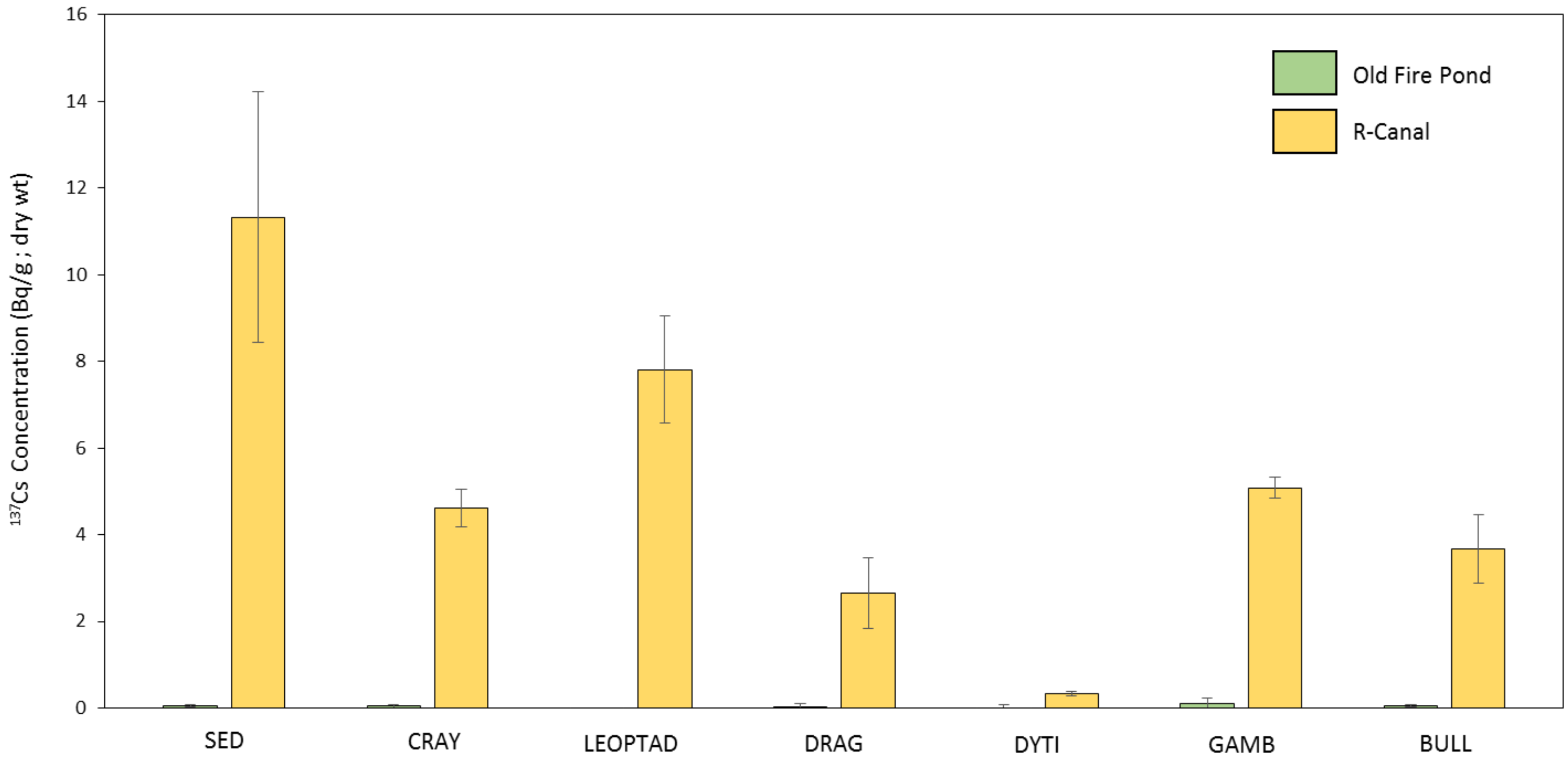

Figure 2.2: Comparison of ^{137}Cs concentrations (Bq/g; dry wt) with 95% Confidence Intervals between biota collected from Old Fire Pond (reference site) and R-Canal (contaminated site) on the U.S. Department of Energy Savannah River Site in Aiken, SC, USA. * *SED Sediment*, *CRAY* crayfish (*Procambarus clarkia*), *LEOPTAD* leopard frog tadpole (*Lithobates spenocephalus*), *DRAG* dragonfly naiads (Family *Libellulidae*), *DYTI* dytiscid beetles (Family *Dytiscidae*), *GAMB* mosquitofish (*Gambusia holbrooki*), *BULL* bullfrog

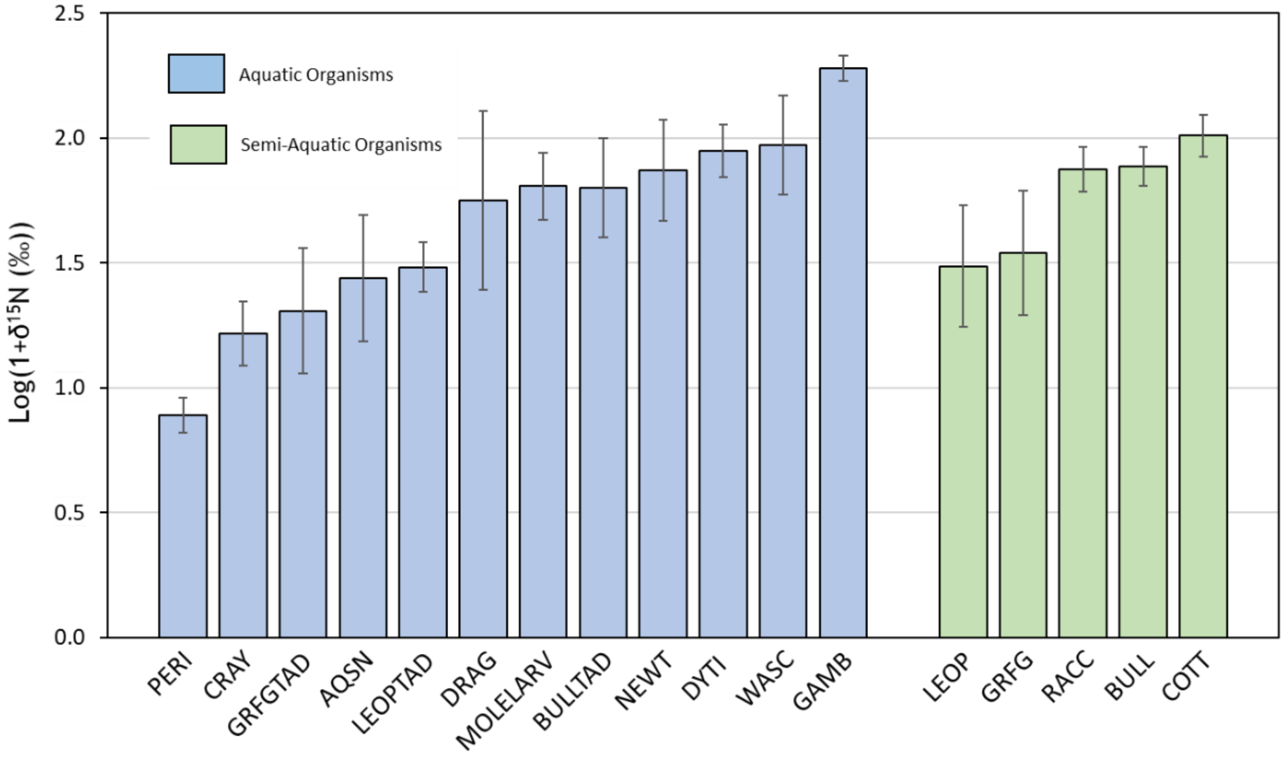

Figure 2.3: Comparison of relative trophic position in aquatic and semi-aquatic biota as a measure of $\delta^{15}N$ (+/- 95% Confidence Intervals) from R-Canal on the U.S. Department of Energy Savannah River Site in Aiken, SC, USA. **PERI* periphyton, *CRAY* crayfish (*Procambarus clarkia*), *GRFGTAD* green frog tadpole (*Lithobates clamitans*), *AQSN* aquatic snails (Family *Planorbidae*), *LEOPTAD* leopard frog tadpole (*Lithobates spenocephalus*), *DRAG* dragonfly naiads (Family *Libellulidae*), *MOLELARV* mole salamander larvae (*Ambystoma talpoideum*), *BULLTAD* bullfrog tadpole (*Lithobates catesbeianus*), *NEWT* red spotted newt (*Notophthalmus viridescens*), *DYTI* dytiscid beetles (Family *Dytiscidae*), *WASC* water scorpion (Family *Nepidae*), *GAMB* mosquitofish (*Gambusia holbrooki*), *LEOP* leopard frog, *GRFG* green frog, *RACC* raccoon (*Procyon lotor*), *BULL* bullfrog, *COTT* cottonmouth (*Agkistrodon piscivorus*)

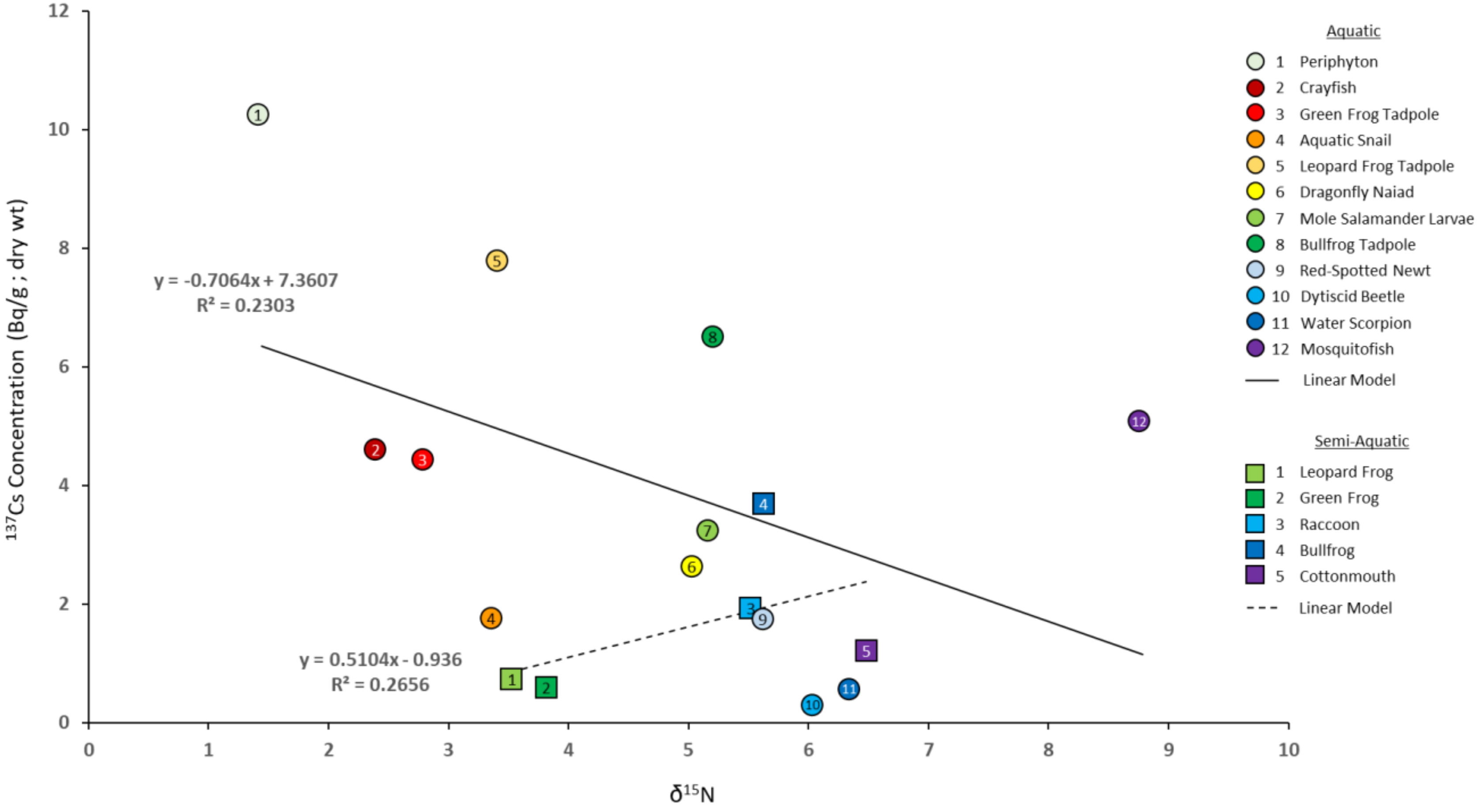

Figure 2.4: Relationship between ^{137}Cs concentrations (Bq/g; dry wt) and estimated nitrogen stable isotope values for aquatic and semi-aquatic biota collected from R-Canal on the U.S. Department of Energy Savannah River Site in Aiken, SC, USA.

CHAPTER 3

INFLUENCE OF ONTOGENETIC SHIFT IN FORAGING STRATEGY AND DIET ON RADIOCESIUM ACTIVITY IN COTTONMOUTHS (*AGKISTRODON PISCIVORUS*) AND THREE SPECIES OF ANURANS INHABITING A CONTAMINATED AQUATIC SYSTEM

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ABSTRACT

Found within many aquatic ecosystems worldwide as a result of anthropogenic pollution from nuclear activities, radiocesium (^{137}Cs) readily bioaccumulates within tissues of exposed biota. Although many factors can influence ^{137}Cs bioaccumulation, ontogenetic shifts in foraging strategies and diet can contribute to differences in ^{137}Cs activity among organisms. However, extensively studied in fish species, the extent to which ontogenetic shifts contribute to variability in ^{137}Cs activity is poorly understood for many organisms. In this chapter, we investigated whether shifts in diet and foraging strategy influence ^{137}Cs activity in several herpetofauna species inhabiting an aquatic ecosystem contaminated with ^{137}Cs on the U.S. Department of Energy's Savannah River Site. We collected pre- and post-metamorphic leopard frogs (*Lithobates sphenocephalus*), green frogs (*L. clamitans*), and bullfrogs (*L. catesbeianus*), as well as juvenile and adult cottonmouths (*Agkistrodon piscivorus*), from a contaminated effluent canal, and quantified whole-body ^{137}Cs concentrations. From these data, we compared ^{137}Cs concentrations between life stages for each species, and examined possible correlations between body size and ^{137}Cs activity for post-metamorphosed bullfrogs and cottonmouths. Our results revealed that ^{137}Cs concentrations in the tadpoles of all three species sampled were higher than concentrations observed in post-metamorphic frogs of the same species, and that ^{137}Cs in post-metamorphosed bullfrogs was negatively correlated with increasing body size. In contrast, ^{137}Cs activity levels did not differ between juvenile and adult cottonmouths, nor was there a significant relationship between ^{137}Cs activity and body length. Although there was no difference in ^{137}Cs activity between juvenile and adult cottonmouths, our findings suggest that ontogenetic

shifts in morphological, phenotypical, and behavioral characteristics between pre- and post-metamorphic stages, and associated changes in body size, can have drastic impacts on ^{137}Cs accumulation in anurans inhabiting polluted environments. Collectively, our findings provide further evidence of the complex biotic and abiotic factors influencing the accumulation of ^{137}Cs within organisms at different life stages, and suggest generalization of ontogenetic accumulation patterns among disparate taxa may not always be appropriate or advisable.

INTRODUCTION

Due to nuclear fallout from weapons testing and nuclear accidents, anthropogenic radionuclides (e.g., radiocesium; ^{137}Cs) are distributed throughout terrestrial and aquatic ecosystems across the world (UNSCEAR 2008, Kawamura et al. 2011, Prăvălie 2014, Steinhauser et al. 2014). There are currently nuclear energy facilities in at least 30 countries worldwide (IAEA 2017), and as human dependence in nuclear energy continues to grow, there remains risk of accidental releases of radionuclide contamination into the environment. Once released, radionuclides can concentrate within the sediments of contaminated ecosystems and accumulate in exposed wildlife species via multiple exposure pathways (Garten et al. 2000).

Given its similar chemical properties to potassium (K), ^{137}Cs readily accumulates in skeletal muscle and other soft tissues of exposed biota, and can be influenced by the availability of K within the environment (Whicker et al. 1990, Paller et al. 2014, Pinder et al. 2014). However, accumulation of radionuclides in general can be highly variable among different biota depending on the behavioral traits of the species, physical characteristics of the contaminated system, and seasonal changes within the environment that affect the availability of the contaminant (Friday 1998). Additionally, ^{137}Cs uptake in exposed biota can be influenced by their life-stage and the associated ontogenetic shifts in morphological, physiological, and behavioral characteristics between juveniles and adults, such as body size, metabolism, foraging behavior, and diet (Carlsson and Lidén 1978, Rowan et al. 1998, Peters and Brisbin 1996). For example, in some species of fish, ^{137}Cs activity is higher in adults compared to juveniles due to a shift from a planktivorous to piscivorous diet and increase in body size as they age (Carlsson and Lidén 1978, Rowan et al. 1998). Similarly, in yellow-bellied slider turtles (*Trachemys scripta*), adults exhibit higher ^{137}Cs activity compared to juveniles due to differences in metabolic rates, as well as possible shifts in diet from invertebrates to plants as the turtle matures (Hart 1983; Peters and Brisbin 1996). Although differences in ^{137}Cs activity between juveniles and adults have been studied in ectothermic biota such as fish and turtles, data for other species with distinctive ontogenetic shifts, such as anurans and snakes, are lacking.

An important taxonomic group in aquatic ecosystems (Holomuzki et al. 1994; Wissinger et al. 1999), amphibians have been found to readily accumulate ^{137}Cs (Matsusshima et al. 2015; Takahara et al. 2015) and thus represent a potential exposure pathway for predators consuming ^{137}Cs exposed individuals. Furthermore, given the high emergent rates of amphibians from wetlands during each breeding season (Gibbons et al. 2006), newly metamorphosed frogs likely contribute to the transport of ^{137}Cs among habitats as they disperse from aquatic systems into adjacent terrestrial environments. As pre-metamorphic juveniles, most amphibian species are confined to aquatic systems, where they forage and grow until they transform to adults that can venture into adjacent terrestrial habitats. With this restriction in habitat, differences in the diet of anurans has been well documented between tadpole and frog life stages (Jenssen 1967, McKamie and Heidt 1974; Werner *et al* 1995, Ruibal and Laufer 2012). As tadpoles, anurans consume an herbivorous diet consisting mainly of algae and decaying plant matter, as well as incidental sediment (Jenssen 1967, Ruibal and Laufer 2012). Once they metamorphose, species such as bullfrogs (*Lithobates catesbeianus*) are voracious predators with a carnivorous diet that consists of variety of prey from invertebrates to small mammals (McKamie and Heidt 1974; Werner *et al* 1995). This dramatic shift in morphology, foraging strategy, and diet associated with metamorphosis has the potential to contribute to widely disparate ^{137}Cs activity among life stages.

Similar to amphibians, ontogenetic shifts in diets and behavior of snakes inhabiting contaminated ecosystems also may result in disparate ^{137}Cs activity between juvenile and adult life stages. Gape-limited to the prey they can physically consume, many species of snakes exhibit a substantial shift in prey selection as they grow (Allen and Swindell 1948, Wharton 1960, Heatwole and Davison 1976). However, differences in morphological and behavioral characteristics can also influence foraging strategies and play a role in prey selection. Especially prevalent in pit vipers, such as cottonmouths (*Agkistrodon piscivorus*), juveniles possess a bright, yellow-colored tail that they use to attract prey in a behavior known as caudal luring (Wharton 1960; Heatwole and Davison 1976). Unlike the adults that are more likely to actively search for prey within the aquatic and adjacent terrestrial habitat, juvenile cottonmouths typically utilize an ambush foraging strategy near the edge of the aquatic habitat while caudal luring, limiting their diet to small organisms such as paedomorphic and newly metamorphosed amphibians

(Eskew et al. 2009). As these snakes mature, they lose the modified coloration in their tails, and often undergo a shift in diet from small amphibians to any prey small enough to capture and consume (Allen and Swindell 1948, Wharton 1960, Heatwole and Davison 1976, Eskew et al. 2009).

Given the widespread disparity in diet and foraging strategy exhibited by many vertebrates as they transition among life stages or age classes, there is a need to better understand how ontogenetic shifts in these behaviors influence the accumulation of ^{137}Cs and other contaminants. In this study, we compared ^{137}Cs concentrations between the pre- and post-metamorphic life stage of three anuran species, as well as between juvenile and adult cottonmouths, an aquatic pit viper, collected from a contaminated effluent canal on the U.S. Department of Energy's Savannah River Site (SRS). Our objectives were to 1) compare differences in ^{137}Cs activity between different life stages of anurans and cottonmouth snakes, and to 2) determine how body size correlates with ^{137}Cs activity in post-metamorphosed bullfrogs and cottonmouths. Because ^{137}Cs was primarily distributed in the sediments within the effluent canal and adjacent floodplain at our study site, we hypothesized that ^{137}Cs activity would be higher in tadpoles and juvenile snakes given their closer association with the sediments and prey within the contaminated canal, and ^{137}Cs concentrations would decrease with increasing body size due to the ability of post-metamorphosed bullfrogs and adult cottonmouths to forage in adjacent, uncontaminated habitats.

METHODS

Study Site

This research was carried out on the U.S. Department of Energy's Savannah River Site (SRS). The historic purpose of the SRS was to produce basic materials necessary for the production of nuclear weapons (Savannah River Nuclear Solutions, LLC 2011). The SRS has since shifted its focus to managing a portion of the U.S. nuclear weapons stockpile and materials, and conducting environmental studies and remediation (White and Gaines 2000). Approximately 800 km^2 in size, the SRS landscape is comprised of both upland pine and bottomland hardwood forests, as well as a variety of wetland, reservoir, and riparian habitats (White and Gaines 2000). As a result of the operation of nuclear facilities and reactors, ^{137}Cs, a product of nuclear fission, was released into four major aquatic systems on the SRS. In particular, the Par Pond-Lower Three Runs system received approximately 8.2×10^{12} Bq of ^{137}Cs into R-Canal from R-Reactor between 1954 and 1964 (Carlton et al. 1992). Consequently, R-Canal is currently the most contaminated region within the Par-Pond-Lower Three Runs system, with radionuclides occurring predominantly confined to the sediments. Past studies examining ^{137}Cs activity within R-Canal reported average (± SE) ^{137}Cs levels in sediments of 11.32 ± 1.47 Bq/g (see Chapter 2). Because it provides habitat for a variety of aquatic organisms and semi-aquatic wildlife species, we sampled along a 250-m segment of R-Canal before it reaches Pond A that was approximately 10 m wide and varied in water depth. The SRS supports abundant herpetofauna within its numerous aquatic and terrestrial habitats, including 27 species of frogs and toads and 36 species of snakes (Wike et al. 2006), many of which can be found in or near R-Canal.

Study Design

During the spring and summer of 2015 and 2016, we collected juvenile and adult anurans and viperid snakes, specifically leopard frogs (*L. sphenocephalus*), green frogs (*L. clamitans*), bullfrogs, and cottonmouths, to quantify whole-body ^{137}Cs activity. Anuran tadpoles were captured using partially submerged minnow traps placed along multiple transects in R-canal, and transferred to sealable bags filled with water from the canal for transport back to the lab. We captured metamorphosed frogs via incidental trapping in minnow traps, or by net and hand sampling along the canal edge. Upon capture, all frogs were placed in sealable containers in the field and transported live to the lab. We also collected cottonmouths ranging in size and maturity from R-Canal and the immediately adjacent areas via hand capture using snake tongs (Gentile Giant 52 Inch Snake Tongs; Midwest Tongs, Greenwood, MO), and placed them in cloth bags for transport back to the lab. Cottonmouths were considered juveniles if they still possessed their yellow tail used for caudal luring. Both frogs and snakes were only collected for this study if they were observed within 20 meters from the edge of the contaminated canal. All animal capture, handling, and euthanasia were carried out in compliance with the University of Georgia Institutional Animal Care and Use Committee (IACUC) guidelines under protocols A2015 12-009-Y2-A3 and A2015 12-017-Y2-A2, and under South Carolina DNR collection permits #05-2015, #F-15-16, #02-2016, and #F-16-05.

Sample Treatment and Analysis

Once brought to the lab, all specimens were euthanized, measured for total body length (to the nearest millimeter) and mass (in grams), and frozen at -20°C. All specimens were later lyophilized, re-weighted to obtain a dry weight (g), and whole-body homogenized for sample preparation and analysis. In its homogenized, powdered form, 0.5 - 2.0 g of each sample (depending on body size) was packed into separate 3.0 mL scintillation vials to be analyzed for ^{137}Cs.

^{137}Cs concentrations were measured using a Packard Cobra II Auto-Gamma Counter (Model Cobra II 5003) with a single 3-inch through-hole NaI detector set to measure ^{137}Cs emissions at 662 kiloelectron-Volts (keV) photons with a counting region of interest between 580-754 keV. The Auto-Gamma Counter was auto-calibrated daily using a traceable sealed source of radiocesium and programmed to analyze each sample for 3,600 seconds, with empty background scintillation vials every fifth sample. Average count yield values were determined using prepared chicken breast spiked with 745 Becquerels (Bq) of ^{137}Cs as standards (standard weights at 1-4 g, in 1 g increments), while minimum detectable concentrations (MDCs) were calculated using methods described by Currie (1968). We then calculated the ^{137}Cs concentration for each sample in Bq per gram dry weight material.

Statistical Analyses

Whole-body ^{137}Cs concentrations were quantified and compared between pre- and post-metamorphosed anurans and between juvenile and adult cottonmouths, for each species collected. Prior to analyses, we evaluated ^{137}Cs data for each species for normality and homoscedasticity using Shapiro-Wilks and Bartlett’s test statistics, respectively, and transformed the data when the residuals were non-normally distributed. Based on these results, we tested for differences in ^{137}Cs activity between life stages for each species using a two-sample T-test or a nonparametric Wilcoxon rank sum test (or Mann-Whitney U-test) for data that were non-normal after log and square root transformations. We further examined the relationship between body size and ^{137}Cs concentrations in post-metamorphosed bullfrogs and cottonmouths using linear regression.

RESULTS

Across our two sampling seasons, we collected between 6 - 20 individuals of both juveniles and adults for each of our target species (Table 3.1). A two sample T-test revealed no difference in ^{137}Cs concentrations between juvenile and adult cottonmouths ($t = -0.5$, $df = 13$, p-value = 0.31; Figure 3.1) collected in or immediately adjacent to R-Canal. In contrast, green frog tadpoles had significantly higher ^{137}Cs activity levels than post-metamorphic green frogs (Log transformed, $t = -7.68$, $df = 19$, p-value < 0.001; Figure 3.2). ^{137}Cs data for both leopard frogs and bullfrogs were non-normal, even after transformations, and thus we tested for differences in ^{137}Cs body activity between life stages using a Wilcoxon rank sum test. Similar to green frogs, both leopard frog tadpoles (p-value < 0.001; Figure 3.3) and bullfrog tadpoles (p-value < 0.001; Figure 3.4) had higher ^{137}Cs concentrations than post-metamorphosed frogs of each species.

In addition to testing for differences in ^{137}Cs concentrations between life stages for all collected species, we also examined the relationship between whole-body ^{137}Cs concentrations and body size for both cottonmouths and post-metamorphosed bullfrogs using linear regression. Given the lack of differences in ^{137}Cs activity between juvenile and adult cottonmouths, both age classes were pooled for this analysis. The results of these models revealed there was no relationship between ^{137}Cs activity and body length (mm) for cottonmouths (Figure 3.5; $R^2 = -0.01$, p-value = 0.37), but there was a significant negative relationship in post-metamorphic bullfrogs (Figure 3.6; $R^2 = 0.72$, p-value < 0.001).

DISCUSSION

Past studies have demonstrated that as aquatic organisms increase in size and maturity, their ^{137}Cs concentrations also increase in response to changes in metabolism, altered food consumption rates and diet, shifts in foraging strategies, and other environmental and biological factors (Carlsson and Lidén 1978, Rowan et al. 1998, Peters and Brisbin 1996). In particular, increases in ^{137}Cs body activity have been documented in several fish species as they grow and experience shifts in diet (Carlsson and Lidén 1978, Rowan et al. 1998). Similarly, elevated ^{137}Cs activity have been observed in adult freshwater turtles compared to juveniles, in part due to dietary shifts and differences in consumption and elimination due to temperature-dependent metabolism (Peters and Brisbin 1996). However, the results of our study suggest organisms exhibit interspecific variability in ^{137}Cs accumulation patterns following ontogenetic shifts in diet and behavior. For example, in contrast to previous observations for fish and turtles, we found higher ^{137}Cs activity in tadpoles compared to post-metamorphic frogs across all three anuran species sampled, and life stage and associated ontogenetic shift in foraging strategy and diet did not influence ^{137}Cs activity in cottonmouths. Furthermore, post-metamorphic bullfrogs exhibited a negative relationship between body size and ^{137}Cs activity, and no relationship was found for cottonmouths. Such variability among species in ontogenetic patterns of ^{137}Cs accumulation likely reflects underlying differences in physiology and behavior, as well as ^{137}Cs bioavailability and environmental characteristics among study systems.

Although comparison of ^{137}Cs contamination levels in both pre- and post-metamorphic life stages of frogs has not been assessed previously, tadpoles have been shown to accumulate higher concentrations of most trace elements compared to post-metamorphic frogs (Roe et al. 2005). Increased contaminant activity in tadpoles likely reflects the propensity for tadpoles to ingest large quantities of contaminated sediments while foraging (Jenssen 1967, Ruibal and Laufer 2012), coupled with the large intestinal surface area relative to body size in tadpoles compared to post-metamorphic frogs (Hourdry et al. 1996, Roe et al. 2005). Within R-Canal, as well as many other contaminated systems on the SRS, the majority of ^{137}Cs is bound within sediments in aquatic systems (Friday 1998), likely contributing to the higher ^{137}Cs contamination activity observed in pre-metamorphic tadpoles. In addition, ^{137}Cs does not appear to biomagnify within our study system (see Chapter 2). Furthermore, ^{137}Cs contamination was mainly restricted within the effluent canal itself where tadpoles were confined (Carlton et al. 1992, Friday 1998), whereas post-metamorphic frogs potentially could have foraged within the adjacent uncontaminated terrestrial habitats, resulting in reduced contaminant activity (Hopkins et al. 1998). Foraging also halts in anuran tadpoles as they undergo physiological and morphological changes during metamorphosis (Hourdry et al. 1996), creating the possibility of contaminant elimination during periods of decreased foraging immediately before exodus from the aquatic system. However, it is unlikely that any of our tadpoles were undergoing this fasting period prior to metamorphosis, as tadpoles developing limbs were excluded from this study. Furthermore, it was unlikely that ^{137}Cs activity in the frogs within this study were affected by this fasting, as all frogs collected had undergone complete tail retention and were of considerable size.

In addition to post-metamorphic frogs having decreased ^{137}Cs activity compared to tadpoles in all three anuran species, post-metamorphic bullfrogs also had ^{137}Cs concentrations inversely related to body length, where larger individuals tended to have reduced contaminant activity. These findings were similar to those from another study conducted on the SRS, where Green treefrogs (*Hyla cinerea*) had whole body ^{137}Cs concentrations negatively correlated with body length and weight (Dapson and Kaplan 1975). This decreased ^{137}Cs activity in bullfrogs in our study may be attributed to decreasing ^{137}Cs exposure through diet, physiology and foraging location as the animals grows. As some species of frogs grow and mature, they begin to venture further from the aquatic system while smaller individuals remain closer to the edge of the water (Bateman and Fleming 2014). Our observations support this finding, as most larger bullfrogs were observed foraging at greater distances from the effluent canal. Because bullfrogs are voracious predators that consume a wide variety of prey, including other frogs (McKamie and Heidt 1974; Werner et al. 1995; Boelter et al. 2012), the gradient of ^{137}Cs contaminated prey correlated with distance from the contamination source is likely to be one of the many contributors to the differences in ^{137}Cs activity in bullfrogs of varying sizes for our study. Unlike frogs on the SRS, ^{137}Cs concentrations in Tago's brown frogs (*Rana tagoi tagoi*) in the Fukushima Prefecture in Japan did not decrease with increasing body size, but instead demonstrated an insignificant positive trend with body size (Takahara et al. 2015). Collectively, these data reinforce the fact that a complex myriad of biotic and abiotic factors contribute to variance in ^{137}Cs accumulation patterns, and thus research investigating ^{137}Cs accumulation among numerous contaminated systems, as well as a variety of taxa, are needed to better elucidate the underlying factors contributing to this variation.

While our study found differences in ^{137}Cs whole-body concentrations between pre- and post-metamorphic anurans, this was not found to be the case for cottonmouths, despite previously reported differences in foraging strategies and diet between juveniles and adults (Heatwole and Davison 1976; Eskew et al. 2009). These findings differ from studies examining the accumulation of other classes of contaminants (i.e.; organochlorides and mercury) in water snakes (including cottonmouths), in which contaminant activity were shown to increase with increasing body size (Santos et al. 1999; Rainwater et al. 2005; Drewett et al. 2013). Elevated organochloride and mercury burdens with increasing size may be in part due to the biomagnification of these contaminants and associated shifts in trophic position as the snakes grow. However, ^{137}Cs does not appear to biomagnify within our study system, and thus ^{137}Cs levels in cottonmouths are not always reflective of their potential prey (see Chapter 2).

The similarity in ^{137}Cs activity between juvenile and adult cottonmouths for our study suggests mechanisms other than diet may be driving ^{137}Cs accumulation in cottonmouths in ^{137}Cs contaminated ecosystems. Differences in metabolic rates between juveniles and adults may influence ^{137}Cs kinetics in cottonmouths, as factors influencing metabolism have been shown to influence ingestion and elimination rates in other ectothermic reptiles (Peters and Brisbin 1996). Furthermore, mechanisms of ^{137}Cs transfer or elimination, such as maternal transfer or depuration through ecdysis (shedding of skin), may also influence ^{137}Cs activity observed in cottonmouths, as has been found to be the case with other contaminants (Linder and Grillitsch 2000; Hopkins et al. 2001, 2004). However, further studies are needed to confirm this. Therefore, future studies involving ^{137}Cs accumulation in cottonmouths of different sizes or life stages should examine additional parameters, both biotic and abiotic, to elucidate possible reasons behind the similarity in ^{137}Cs activity between life stages and across sizes observed in our study.

To our knowledge, our study is the first to compare ^{137}Cs whole-body concentrations between pre- and post-metamorphic anuran life stages, and juvenile and adult viperid snakes. To understand the bioaccumulation of contaminants within biota through aquatic and terrestrial ecosystems, it is important to elucidate how ontogenetic shifts in morphological, dietary, and behavioral characteristics can influence ^{137}Cs activity across many different species. Our study showed clear distinction between ^{137}Cs activity in tadpoles and frogs, while juvenile and adult cottonmouths possessed similar levels of contamination, demonstrating how differences in foraging strategy and diet associated with life stage can influence ^{137}Cs activity depending on the species in question. Future studies measuring ^{137}Cs in herpetofauna should consider testing multiple life stages for several species, as ^{137}Cs accumulation in biota can be highly variable, especially between species or life stages where differences in morphological, behavioral and physiological characteristics can influence ^{137}Cs uptake.

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Table 3.1: Whole body ^{137}Cs concentrations (Bq/g; dry wt) for juvenile and adult anuran and viperid species collected from R-Canal on the Savannah River Site in Aiken, SC, USA. All juvenile anurans were pre-metamorphic.

		Juveniles		Adults
Species	*N*	^{137}Cs Bq/g dry wt $\bar{X}$ ± 1 SE (min/max)	*N*	^{137}Cs Bq/g dry wt $\bar{X}$ ± 1 SE (min/max)
Leopard Frogs (*Lithobates sphenocephalus*)	8	7.81 ± 0.63 (5.43/9.55)	8	0.71 ± 0.28 (0.09/2.24)
Green Frogs (*Lithobates clamitans*)	6	4.48 ± 0.66 (2.25/6.16)	15	0.62 ± 0.10 (0.24/1.38)
Bullfrogs (*Lithobates catesbeianus*)	20	6.21 ± 0.49 (2.76/10.94)	18	2.42 ± 0.33 (0.39/4.15)
Cottonmouths (*Agkistrodon piscivorus*)	6	1.30 ± 0.41 (0.61/3.00)	9	1.11 ± 0.16 (0.64/1.85)

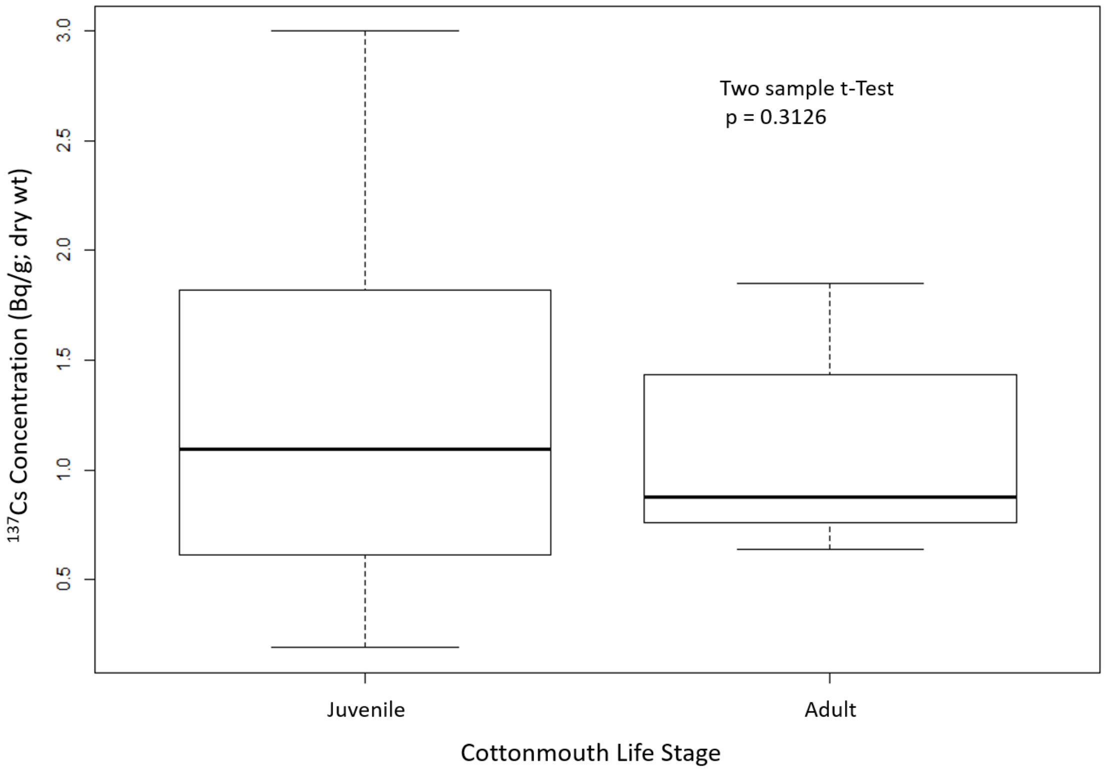

Figure 3.1: Comparison of whole-body ^{137}Cs concentrations between juvenile and adult cottonmouths (*Agkistrodon piscivorus*) collected from a contaminated effluent canal on the Savannah River Site in Aiken, SC, USA.

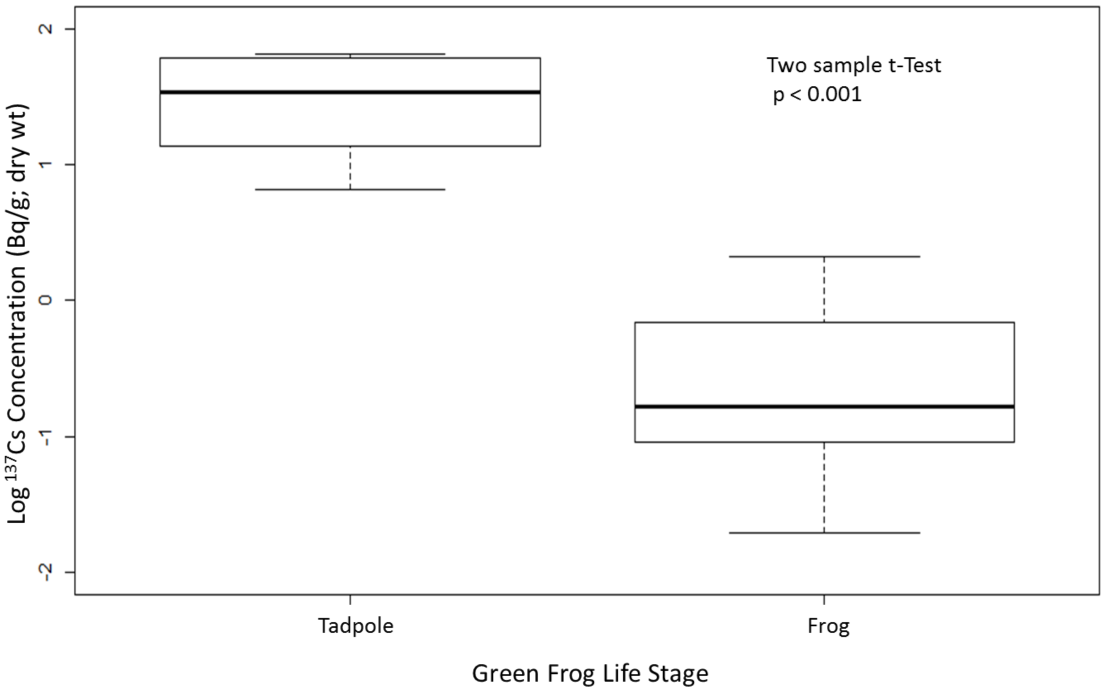

Figure 3.2: Comparison of whole-body ^{137}Cs concentrations between pre- and post-metamorphic life stages (tadpole and frog, respectively) of green frogs (*Lithobates clamitans*) collected from a contaminated effluent canal on the Savannah River Site in Aiken, SC, USA.

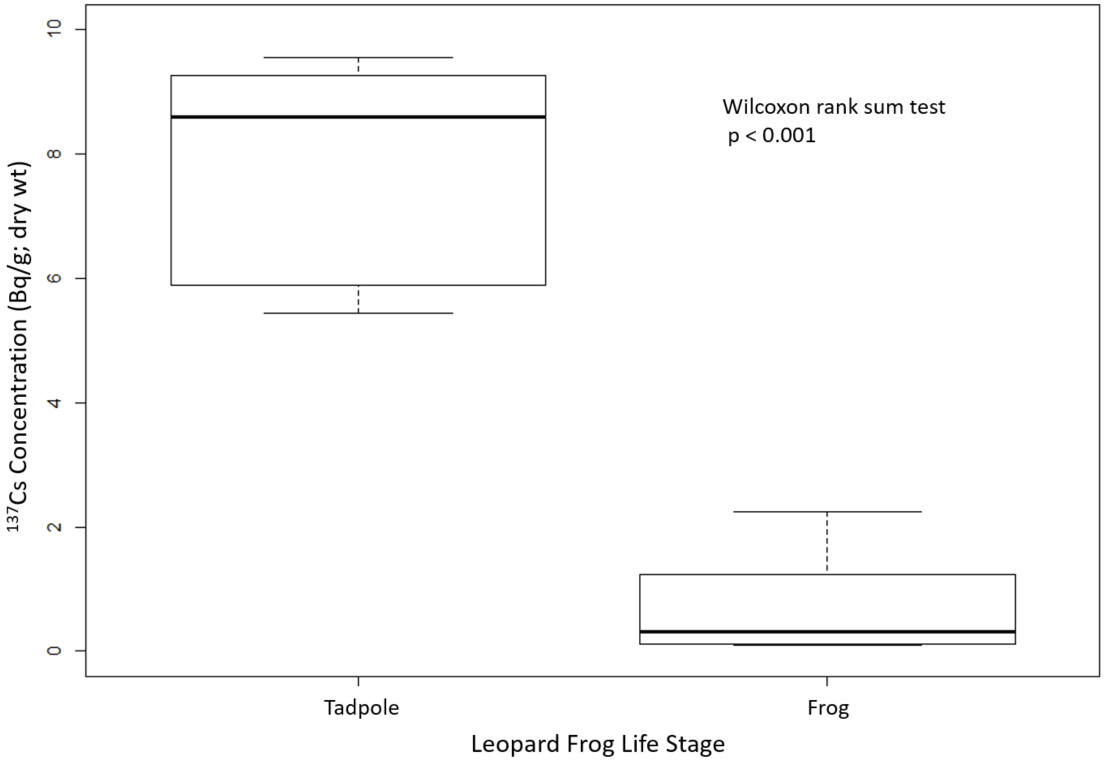

Figure 3.3: Comparison of whole-body ^{137}Cs concentrations between pre- and post-metamorphic life stages (tadpole and frog, respectively) of leopard frogs (*Lithobates sphenocephalus*) collected from a contaminated effluent canal on the Savannah River Site in Aiken, SC, USA.

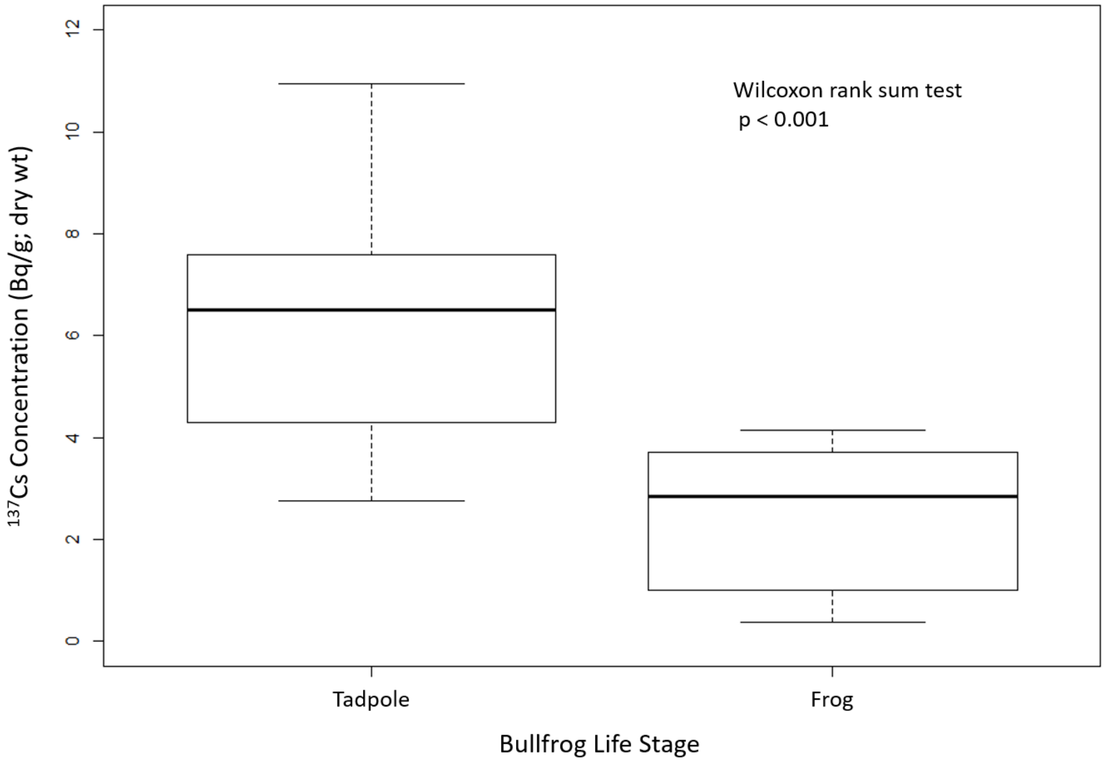

Figure 3.4: Comparison of whole-body ^{137}Cs concentrations between pre- and post-metamorphic life stages (tadpole and frog, respectively) of bullfrogs (*Lithobates catesbeianus*) collected from a contaminated effluent canal on the Savannah River Site in Aiken, SC, USA.

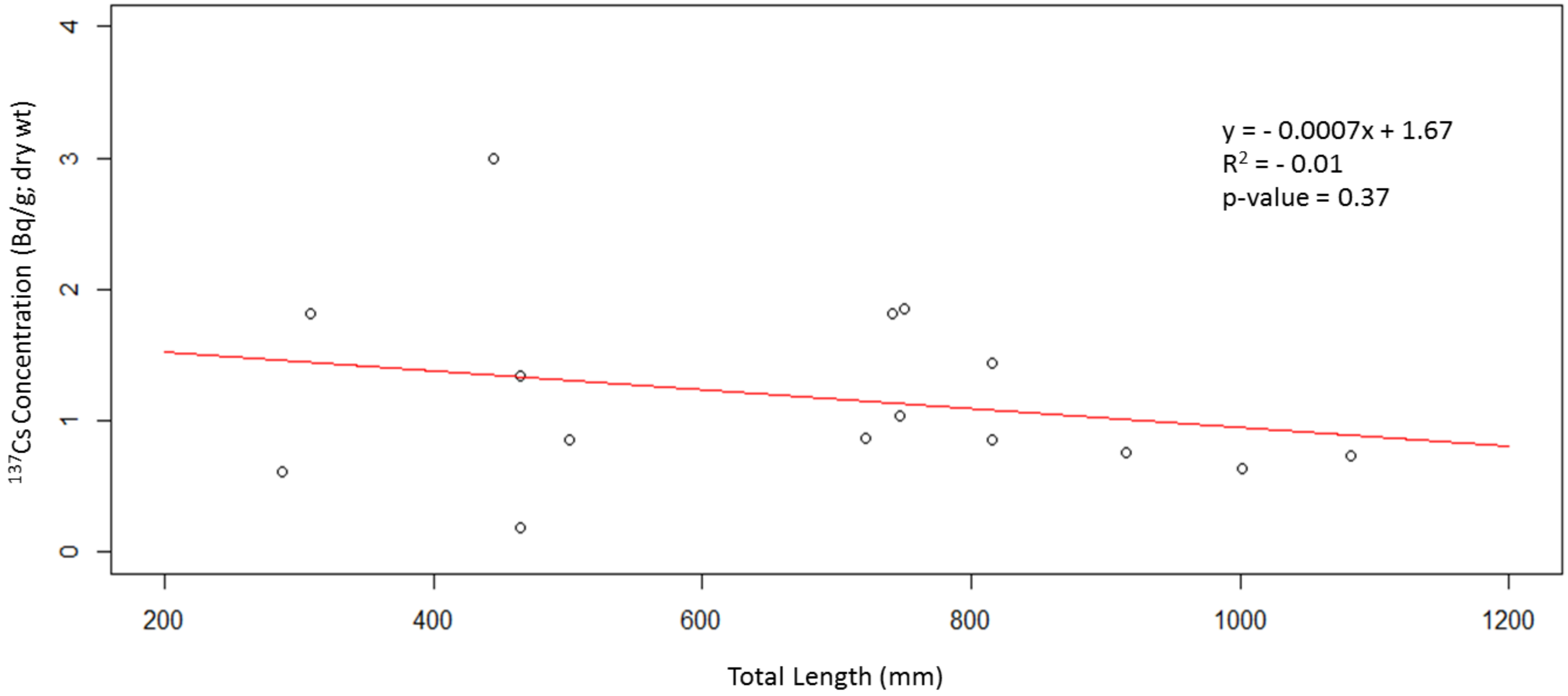

Figure 3.5: Correlation between whole-body ^{137}Cs concentrations and total body length in cottonmouths (*Agkistrodon piscivorus*) collected from a contaminated effluent canal on the Savannah River Site in Aiken, SC, USA.

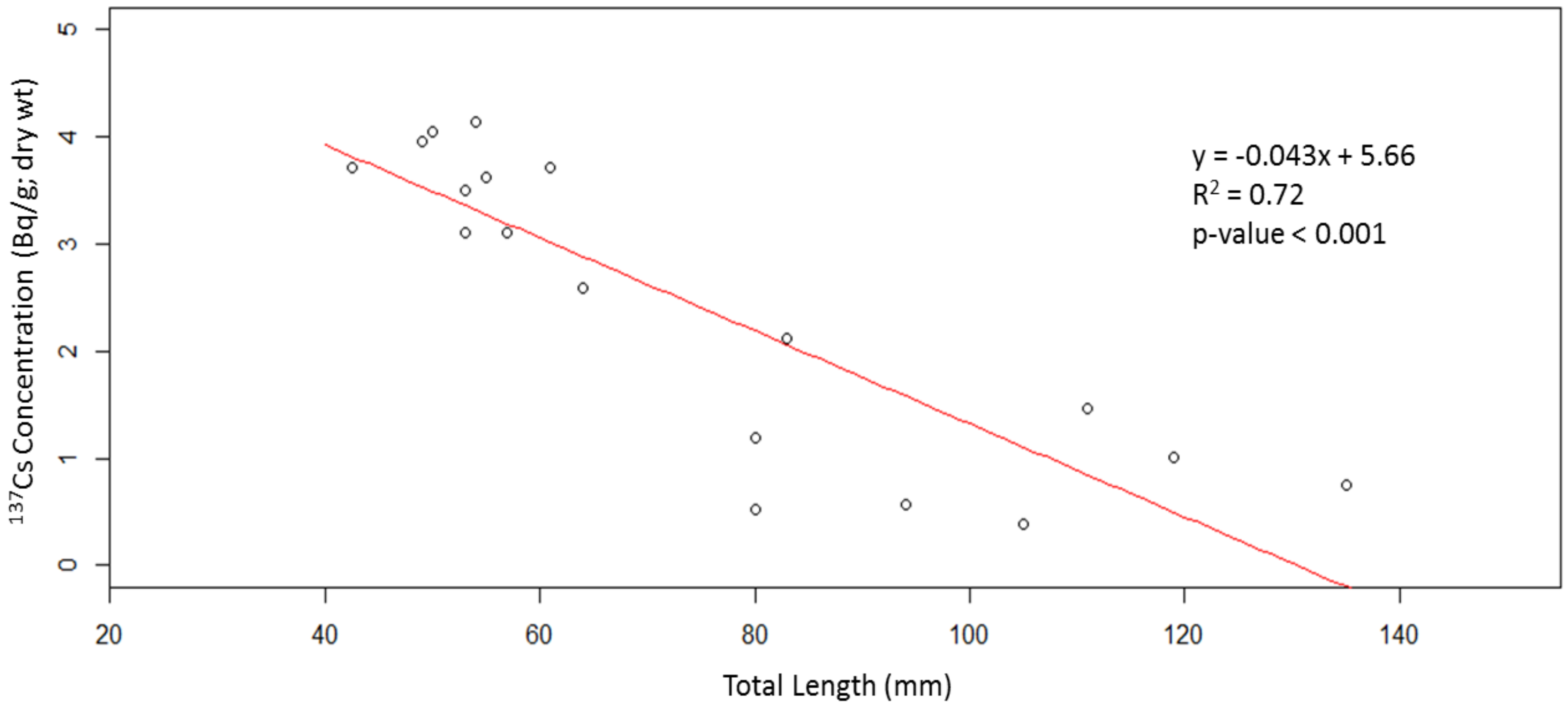

Figure 3.6: Relationship between whole-body ^{137}Cs concentrations and total body length in post-metamorphic bullfrogs (*Lithobates catesbeianus*) collected from a contaminated effluent canal on the Savannah River Site in Aiken, SC, USA.

CHAPTER 4

ACCUMULATION OF RADIOCESIUM IN BULLFROG TADPOLES IN A CONTAMINATED EFFLUENT CANAL ON THE U.S. DEPARTMENT OF ENERGY'S SAVANNAH RIVER SITE

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ABSTRACT

As a result of global and localized distribution from activities such as nuclear weapons testing, faulty power generation and waste disposal, and nuclear disasters, radiocesium (^{137}Cs) is a radio-contaminant of concern that readily accumulates in exposed wildlife. Although bioaccumulation of ^{137}Cs is an important factor for understanding its fate within the environment, research examining ^{137}Cs uptake in exposed biota is only available for a limited number of species. In particular, currently there are no data available on bioaccumulation patterns of ^{137}Cs in amphibians, despite their widespread distribution and potential to transport contaminants between aquatic and terrestrial ecosystems. Therefore, the objective of this study was to determine the amount of time necessary for anuran larvae experimentally placed in a contaminated system to reach a steady-state whole-body ^{137}Cs concentration, and to determine the threshold at which that steady-state ^{137}Cs concentration occurred for tadpoles within our study system. By restricting uncontaminated bullfrog (*Lithobates catesbeianus*) tadpoles to three experimental enclosures located along a ^{137}Cs contaminated effluent canal on the U.S. Department of Energy's Savannah River Site, we modeled ^{137}Cs uptake through time using the von Bertalanffy modification of the Richards model. We found that ^{137}Cs accumulation in tadpoles differed slightly among our sampling sites, and thus we constructed separate Richards models for each site. The results of our Richards models indicated that bullfrog tadpoles achieved steady-state ^{137}Cs concentrations of 3.68-4.34 Bq/g ^{137}Cs dry whole body weight after 11.63-15.50 days of exposure among sampling sites, with an average of 3.91 Bq/g after 14.02 days exposure. Radiocesium accumulation in bullfrog tadpoles was found to be more rapid than that reported for other

biota studied from other contaminated systems, likely due to the closer association of tadpoles to sediments, demonstrating the importance of quantifying accumulation rates of ^{137}Cs across a range of biota within contaminated ecosystems.

INTRODUCTION

Environmental contamination by radionuclides is a global phenomenon influenced by anthropogenic sources such as fallout from nuclear weapons testing, faulty nuclear power production and waste disposal, and nuclear accidents (Garten et al. 2000; Prăvălie 2014; Steinhauser et al. 2014). With more than 450 nuclear reactors currently in operation or construction across 30 countries around the globe (IAEA 2017), there is an associated risk of accidental introduction of radiocontamination into the environment. Although decades of nuclear weapons testing and two major nuclear accidents – the Chernobyl nuclear disaster in 1986 and the Fukushima-Daiichi disaster in 2011 – have spread nuclear contamination on a global scale (Prăvălie 2014; Steinhauser et al. 2014), environmental contamination with radionuclides resulting from nuclear production is more localized (Garten et al. 2000). In particular, radiocesium (^{137}Cs), which is produced from nuclear fission, is one of the most widely distributed radionuclides from these anthropogenic activities (Garten et al. 2000; Prăvălie 2014; Steinhauser et al. 2014) and has a physical half-life of 30.2 years. Once introduced into the environment, ^{137}Cs readily enters food webs by accumulating within the soft tissues of exposed organisms and plant cell membranes (Friday et al. 1998; Casadesus et al. 2008; Paller et al. 2014).

Although a number of studies have investigated the fate of ^{137}Cs in the environment, relative levels in exposed biota, and the potential for associated health effects, few studies have investigated bioaccumulation rates in exposed organisms. Of these, most studies have examined ^{137}Cs accumulation rates in birds, turtles, or fish. In particular, given concerns for human exposure through consumption of game meat, ^{137}Cs accumulation rates have been extensively studied in game birds and waterfowl in both controlled laboratory conditions (Anderson et al. 1976, Moss and Horrill 1996), as well as natural settings by restricting individuals to contaminated waterbodies for known periods of time (Fendley et al. 1977, Potter 1987, George et al. 1991, Kennamer et al. 2017). In diet-controlled laboratory studies, experiments examining ^{137}Cs uptake in gallinaceous gamebirds revealed similar accumulation rates among different species, with bobwhite quail (*Colinus virginianus*) reaching a steady-state ^{137}Cs equilibrium after 21 days of dietary exposure (Anderson et al. 1976), and red grouse (*Lagopus lagopus scotica*) after 20-23 days (Moss and Horrill 1996). However, waterfowl exhibit substantial among-species variation in ^{137}Cs bioaccumulation rates when naturally exposed to ^{137}Cs in contaminated waterbodies. For example, wood ducks (*Aix sponsa*; Fendley et al. 1977), ring-necked ducks (*Aythya collaris*; Kennamer et al. 2017), and mallards (*Anas platyrhynchos*; George et al. 1991) restricted to contaminated waterbodies on the U.S. Department of Energy's Savannah River Site (SRS) reached maximum whole-body ^{137}Cs concentrations in 17, 90, and 175 days of exposure, respectively. This disparity in the amount of time necessary to reach steady-state ^{137}Cs equilibrium among species likely reflects differences in diet, foraging strategies, metabolism, and habitat use, as well as other potential biotic and abiotic factors.

Similarly, accumulation rates of ^{137}Cs in yellow-bellied slider turtles (*Trachemys scripta*) were found to be dependent upon ^{137}Cs kinetics, specifically the influence of temperature-dependent metabolism on food ingestion and elimination rates (Peters and

Brisbin 1996). Furthermore, statistical modeling of ^{137}Cs uptake in fish indicated variable accumulation patterns in grunts (*Jarbua terapon*), where accumulation to steady-state ^{137}Cs activity concentrations varied between 37 and 80 days depending on somatic growth rate (Pan and Wang 2016). Collectively, these studies demonstrate that ^{137}Cs accumulation rates can be highly variable between different species of organisms, prompting the need for further research to elucidate ^{137}Cs uptake rates in biota often exposed to ^{137}Cs in contaminated environments.

Often considered keystone species in many ecosystems (Holomuzki et al. 1994; Wissinger et al. 1999), amphibians have the potential to play an important role in influencing energy, nutrient, and contaminant dynamics within many aquatic systems (Gibbons et al. 2006; Unrine et al. 2007). Amphibians readily accumulate ^{137}Cs during their pre- and post-metamorphic life stages when inhabiting contaminated ecosystems (Matsusshima et al. 2015; Takahara et al. 2015). Although adverse health effects and developmental impairment may occur with high dose radiation exposure in contaminated ecosystems, amphibian larval have been found to be quite tolerant of ^{137}Cs exposure with no effects on growth or development in low dose (up to 222 mGy d^{-1}) experimental studies (Stark et al. 2012). Contamination with ^{137}Cs is a potential concern in larval amphibians due the possible transfer of radionuclides from aquatic to terrestrial food webs after metamorphosis, as hundreds of thousands of amphibians can metamorphose from a single wetland after the breeding season (Gibbons et al. 2006). Because of this potential to move ^{137}Cs contamination into food webs overlapping with uncontaminated habitats, understanding the rate at which ^{137}Cs accumulates in amphibian larvae is crucial for understanding the fate and transport of ^{137}Cs from contaminated environments.

Given the limited data on ^{137}Cs accumulation rates in biota, and considerable variance in accumulation patterns among previously evaluated species, our objective in this study was to determine the rate at which amphibian larvae inhabiting a contaminated aquatic ecosystem accumulate ^{137}Cs. We quantified the rate of ^{137}Cs accumulation by placing bullfrog tadpoles (*Lithobates catesbeianus*) within experimental enclosures positioned within a contaminated effluent canal on the SRS, and sampled individuals over 32 days of exposure. We selected bullfrogs as our model organism due to their widespread invasive distribution in many suitable wetland ecosystems across the globe (Ficetola et al. 2007). We hypothesized that bullfrog tadpoles would accumulate ^{137}Cs more quickly than previously studied species (e.g., birds, turtles, and fish) given their closer association with contaminated sediments within the ecosystem.

METHODS

Study Site

The SRS is a U.S. Department of Energy superfund site containing localized environmental contamination resulting from nuclear activities to produce materials for nuclear weapons beginning in the early 1950's (Carlton et al. 1992). The SRS has restricted access to its approximately 800 km^2 landscape, which consists of upland pine and bottomland hardwood habitats, with numerous interspersed permanent and semi-permanent wetlands, creeks and reservoirs (White and Gaines 2000). Contaminated by the SRS R-Reactor between 1954 and 1964, the Lower Three Runs system received approximately 8.2 x 10^{12} Bq of ^{137}Cs into the aquatic environment (Carlton et al. 1992). This contamination was directed into a shallow canal (known as R-Canal) near the reactor beginning in 1954, and environmental contamination of ^{137}Cs continued until

1964, when the reactor was decommissioned (Carlton et al. 1992). Radiocesium is still present within the sediments in R-Canal, and continues to accumulate in exposed biota within the system (see Chapters 2 and 3).

Field Methods

To quantify bioaccumulation of ^{137}Cs in bullfrog tadpoles, we constructed three experimental enclosures and placed them within R-Canal on the SRS. Enclosures were 2.5 x 1.25 x 0.5 meters in size, and placed approximately 100 meters apart along the canal in locations where they were at least 75% submerged at any given point in time, but never completely submerged (Figure 4.1). All enclosures consisted of a wooden frame covered by 0.635 cm^2 mesh metal hardware cloth with a large, hinging top panel for interior access. All enclosures were deployed into R-Canal 30 days prior to stocking with tadpoles, allowing each to accumulate algae and periphyton.

In June 2017, we stocked each enclosure with 35 bullfrog tadpoles that were either collected from an uncontaminated wetland on the SRS a week prior to this study, or raised in mesocosms from eggs collected the previous year. Raised tadpoles were hatched from egg masses collected from an uncontaminated wetland on the SRS. Egg masses were transferred to a mesocosm with decaying plants, leaves, and algae located at the Savannah River Ecology Lab. Prior to releasing tadpoles into our experimental enclosure, 20 tadpoles (10 raised, and 10 captured) were sacrificed to determine background levels of ^{137}Cs. After releasing tadpoles into our enclosures, we collected three individuals from each enclosure every 48 h for 12 days, and then again at 20 and 32 days exposure. All tadpoles were deployed around the same stage of anuran development (Gosner stage 25; Gosner 1960). Bullfrog tadpoles were collected from each enclosure using dip-nets and were placed into water-filled containers for transport to the Savannah River Ecology Laboratory, where they were then euthanized via submersion in tricaine methanesulfonate (MS-222). All tadpoles were cared for and handled in accordance to IACUC protocol A2015 12-017-Y2-A2, and under South Carolina DNR collection permit #SC-04-2017.

Laboratory Methods

Once at the lab, all tadpoles were euthanized, measured for total body length (to the nearest millimeter), and weighed (g). The entire digestive tract of each tadpole was removed through dissection prior to weight determination and rinsed with deionized water in order to purge the gut of any sediments or dietary content. Clean digestive tracts were returned to each tadpole before each individual was weighed and frozen at -20°C until further processing. We later lyophilized our samples via freeze drying, reweighed them to obtain a dry weight, and homogenized each individual bullfrog tadpole sample using mortar and pestle, before packing each into separate 3.0 mL scintillation vials to be analyzed for ^{137}Cs.

Radiocesium concentrations were measured using a Packard Cobra II Auto Gamma Counter (Model Cobra II 5003) with a single 3-inch through-hole NaI detector set to measure radiocesium emissions at 662 kiloelectron-Volts (keV) photons, with a counting region of interest between 580-754 keV. We auto-calibrated the Auto-Gamma Counter daily using a traceable sealed source of radiocesium (SREL sealed source – 01113), and analyzed each sample for 3600 second count intervals with empty, background scintillation vials at every fifth sample. For ^{137}Cs calculations, we determined average count yield values using prepared chicken breast spiked with 745

Becquerels (Bq) of radiocesium as standards (standard weights at 1-4 g, in 1 g increments), while minimum detectable concentrations (MDCs) were calculated using methods described by Curie (1968). We then calculated and standardized ^{137}Cs concentration for each sample in Bq per gram dry weight material.

Statistical Analyses

Prior to analyses, we evaluated all ^{137}Cs data for normality and homoscedasticity using Shapiro-Wilks and Bartlett's test statistics, respectively. To determine whether we could pool tadpoles collected from different enclosures into a single analysis, we examined our data for differences in ^{137}Cs uptake among our enclosure locations. We did this by comparing mean tadpole ^{137}Cs concentrations among our three sites using an Analysis of Variance (ANOVA) followed by a Tukey's HSD Test.

Due to differences in ^{137}Cs concentrations among our three sites at different exposure periods (see results), we modeled ^{137}Cs bioaccumulation separately for each enclosure location. We used the Richards Model modified for contaminant uptake (Potter 1987, Peters and Brisbin 1986) to determine bioaccumulation rates for ^{137}Cs in bullfrog tadpoles at each enclosure, and to determine the amount of time necessary for tadpoles to reach steady-state equilibrium concentrations of ^{137}Cs. The Richards model form used to describe contaminant uptake is defined by the equation:

$$C_t = \ [(C_e^{(1-m)} - \ (C_e^{(1-m)} - \ C_0^{(1-m)})) * \ \exp(\frac{-2t}{T}(m+1))]^{1/(1-m)}$$

where C_t is the concentration of the contaminant in the organism at time t, C_e is the final contaminant concentration at equilibrium (when contaminant uptake is equal to elimination), C_0 is the concentration prior to exposure, t is the exposure period, T is the amount of time for C_t to reach 95% of the maximum concentration at equilibrium, and m

is the Richards shape parameter (Richards 1959; Brisbin et al. 1990). We evaluated fit of several common sigmoidal models (classic Richards model - m = 0, von Bertalanffy - m = 0.67, Gompertz - m = 1.2, Logistic - m = 2; Richards 1959) by changing the constant value for the shape parameter (m). We modeled our data using each of the aforementioned Richards shape parameters within the Richards model for contaminant uptake at each of our enclosure locations, and determined the top-ranking model based on Akaike Information Criterion values corrected for small sample sizes (AICc) and model AICc weights (W). We then used the top-ranking models to estimate what exposure duration was necessary for ^{137}Cs activity in bullfrog tadpoles to reach asymptotic concentrations, and at what concentration steady-state equilibrium of ^{137}Cs was achieved for each sampling location.

RESULTS

With the exception of one of our experimental enclosures (Location A), three tadpoles were collected from each enclosure every 48 h for 12 days, and then again at 20 and 32 days exposure. We were unable to collect any tadpoles from the enclosure at Location A at 20 days post-exposure due to too few tadpoles surviving in the enclosure. We did, however, collect tadpoles from this enclosure at day 32 to carry each sampling location to the end of the study. When testing for differences in ^{137}Cs uptake in bullfrog tadpoles, our ANOVA model indicated there was a significant difference in tadpole ^{137}Cs concentrations among our three enclosure locations (p-value < 0.001). A Tukey's HSD Post Hoc analysis further revealed that ^{137}Cs concentrations in tadpoles from sites A and B were significantly lower than those from site C (A-C, p-value < 0.001; B-C, p-value < 0.001), but were not significantly different from one another (A – B, p-value = 0.84).

Because average uptake of ^{137}Cs concentrations was different among our enclosure locations, we ran separate Richards models for tadpoles collected at each sampling location. For Locations A and B, our AICc results from our four Richards Models indicate that the von Bertalanffy model best fits our data (Location A, Table 4.1; Location B, Table 4.2). Our AICc results for Location C produced two competing models within Δ2AICc units, and thus we selected the top-ranked model – the von Bertalanffy model derived from the Richards Model – to model ^{137}Cs accumulation rates (Table 4.3). We used parameter estimation from these models (Figure 4.2) to determine the amount of time necessary to reach maximum steady-state ^{137}Cs equilibrium concentrations at each enclosure location. Location A reached an average of 3.68 Bq/g ^{137}Cs (dry wt; t-value = 16.99, SE = 0.22) after 14.93 days of ^{137}Cs exposure (t-value = 7.32, SE = 2.04). Similarly, tadpoles collected from Location B reached steady-state ^{137}Cs equilibrium at an average of 3.72 Bq/g ^{137}Cs (dry wt; t-value = 32.77, SE = 0.11) after 15.5 days exposure (t-value = 12.33, SE = 1.26). Finally, bullfrog tadpoles at Location C reached asymptotic ^{137}Cs concentrations of 4.34 Bq/g ^{137}Cs (t-value = 25.89, SE = 0.17) after 11.63 days (t-value = 7.93, SE = 1.47) exposure in R-Canal. Considering the results from the uptake model for each of our three locations, bullfrog tadpoles in R-Canal reached steady-state ^{137}Cs equilibrium at an average of 3.91 Bq/g (dry wt) after 14.02 days of natural ^{137}Cs exposure in the natural environment.

DISCUSSION

Information on accumulation rates of radionuclides in exposed wildlife is crucial for understanding how anthropogenic pollution can potentially influence the health of exposed populations or individuals, as well as advancing our knowledge of the fate and transport of contaminants through ecosystems. Our study is the first to examine accumulation rates of ^{137}Cs in larval amphibians within a contaminated natural system, and from these data we modeled ^{137}Cs uptake to determine the time necessary for bullfrog tadpoles to reach a steady-state, maximum concentration within their tissues. Our findings suggest that after approximately 14 days of exposure, bullfrog tadpoles reached a maximum whole-body ^{137}Cs concentration of approximately 3.91 Bq/g (dry wt) within the R-Canal system on the SRS. These results suggest amphibian larvae are capable of reaching steady-state whole-body ^{137}Cs activity more quickly than other species of wildlife for which accumulation rates of ^{137}Cs have been quantified, such as fish (Pan and Wang 2016), turtles (Peters and Brisbin 1996), and birds (Fendley et al. 1977, Potter 1987, George et al. 1991, Moss and Horrill 1996, Kennamer et al. 2017).

Given the relatively rapid accumulation of ^{137}Cs found within our bullfrog tadpoles compared to other organisms within the current literature, as well as variability in accumulation rates among previously studied species, it is clear that patterns of ^{137}Cs accumulation can be highly variable among species that share similar habitats. Even among waterfowl there is extensive variability in ^{137}Cs accumulation rates; wood ducks reached maximum asymptotic levels of ^{137}Cs in an average of 17.3 days exposure (Fendely et al. 1977), while ring-necked ducks (Kennamer et al. 2017) and mallards (George et al. 1990) took much longer, at approximately 75 and 180 days, respectively. These differences among species within the same family of ducks are likely attributed to various biotic and abiotic factors, such as diet, foraging strategies, and/or habitat use. Likewise, intraspecific variation in ^{137}Cs concentrations among individual yellow-bellied slider turtles have been attributed to differences in temperature, age, diet, metabolism, and consumption and elimination rates (Peters and Brisbin 1996).

Interestingly, we found slightly different ^{137}Cs accumulation rates among sampling sites positioned only 100m apart within our study system. Specifically, we found that tadpoles collected from our sampling site furthest away from R-Reactor reached steady-state equilibrium a few days faster than the other two sampling locations, and accumulated overall higher concentrations of ^{137}Cs. Spatial variation in ^{137}Cs distribution is widely recognized, even within localized areas (Sutherland 1994, Tanaka et al. 2013, Steinhauser et al. 2014). Nonetheless, our data suggest ^{137}Cs bioaccumulation in biota can also be heterogeneous at very small spatial scales, emphasizing the importance of including multiple sampling locations in future field studies assessing contaminant accumulation patterns in natural systems.

The rapid bioaccumulation of ^{137}Cs in bullfrog tadpoles compared to other wildlife species may be partially due to the inherent differences in diet and foraging strategy. Anuran larvae consume decaying plant matter, periphyton, algal biofilms, micro-invertebrates, fungi, and incidental sediments while foraging for food (Jenssen 1967). These dietary items often have high ^{137}Cs activity levels (see Chapter 2, Friday 1998, Murakami et al. 2014), likely facilitating rapid accumulation of high levels of ^{137}Cs. External absorption of ^{137}Cs through the skin may also attribute to some of the activity found within the bullfrog tadpoles in our study, as other studies have shown where an amphibian, the moor frog (*Rana arvalis*), can absorb ^{137}Cs through exposure to contaminated soil during hibernation (Stark et al. 2004). However, the incidental consumption of sediment alone undoubtedly contributes to the rapid accumulation of

^{137}Cs in anuran larvae, as most ^{137}Cs within contaminated systems is concentrated in the sediments (see Chapter 2, Friday 1998). Compared to ^{137}Cs concentrations quantified in bullfrog tadpoles in R-Canal from earlier chapters where gut contents were included in the whole-body ^{137}Cs activity (see Chapters 2 and 3), tadpoles in the present chapter achieved overall lower concentrations of ^{137}Cs. Furthermore, while removing the contents of each tadpole analyzed in this study, each gut was observed to be heavily filled with both the sediments and periphyton that were observed to have the highest ^{137}Cs concentrations of all samples collected from R-Canal (see Chapter 2). This diet, combined with a large intestinal surface area relative to body size in anuran larvae, may enhance contaminant exposure (Hourdry et al. 1996), and cause ^{137}Cs to bioaccumulate quickly within amphibian larvae inhabiting contaminated environments.

Overall, our findings demonstrate the importance of monitoring ^{137}Cs concentrations in amphibian species in ecosystems contaminated from anthropogenic pollution. Although numerous species are exposed to contamination when pollution is released into the environment (Friday 1998), recent studies have recognized the importance of monitoring contaminant levels in anuran species (Matsusshima et al. 2015; Takahara et al. 2015). Larval amphibians can accumulate high levels of ^{137}Cs in contaminated environments due to their high exposure through diet, and can reach maximum levels rather quickly compared to other biota that utilize contaminated aquatic systems. These ^{137}Cs accumulation patterns in amphibians are important to understand and consider given their potential to transfer contaminants within food webs during various stages of their life cycles, as well as disperse ^{137}Cs into terrestrial ecosystems following metamorphosis. Further studies are needed to elucidate the mechanisms behind ^{137}Cs kinetics in amphibians, and to quantify the extent to which amphibians may play a role in the transfer of ^{137}Cs and other contaminants from contaminated aquatic ecosystems to adjacent terrestrial habitats. Furthermore, our findings along with previous research suggests ^{137}Cs accumulation rates are subject to variation between and among exposed wildlife species within different systems, supporting the need for more species to be examined to fully elucidate ^{137}Cs accumulation within exposed biota.

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TABLE 4.1: Rankings of the Richards Model used for fitting contaminant uptake models for estimating the asymptotic concentration of ^{137}Cs in bullfrog tadpoles exposed in Location A in R-Canal on the United States Department of Energy's Savannah River Site, and the exposure time necessary to reach 95% of that maximum concentration using Akaike Information Criterion values corrected for a small sample size (AICc).

Model	Richards Shape Parameter (m)	Log-Likelihood (K)	Number of Parameters	AICc	ΔAICc	Akaike Weight (W)
von Bertalanffy	0.67	-19.97	4	49.05	0.00	0.72
Gompertz	1.2	-21.18	4	51.47	2.42	0.22
Logistic	2	-23.12	4	55.35	6.30	0.03
Classic	0	-23.12	4	55.35	6.30	0.03

TABLE 4.2: Rankings of the Richards Model used for fitting contaminant uptake models for estimating the asymptotic concentration of ^{137}Cs in bullfrog tadpoles exposed in Location B in R-Canal on the United States Department of Energy's Savannah River Site, and the exposure time necessary to reach 95% of that maximum concentration using Akaike Information Criterion values corrected for a small sample size (AICc).

Model	Richards Shape Parameter (m)	Log-Likelihood (K)	Number of Parameters	AICc	ΔAICc	Akaike Weight (W)
von Bertalanffy	0.67	-1.73	4	12.48	0.00	0.77
Classic	0	-3.05	4	15.12	2.64	0.21
Gompertz	1.2	-5.41	4	19.85	7.37	0.02
Logistic	2	-11.06	4	31.14	18.66	0.00

TABLE 4.3: Rankings of the Richards Model used for fitting contaminant uptake models for estimating the asymptotic concentration of ^{137}Cs in bullfrog tadpoles exposed in Location C in R-Canal on the United States Department of Energy's Savannah River Site, and the exposure time necessary to reach 95% of that maximum concentration using Akaike Information Criterion values corrected for a small sample size (AICc).

Model	Richards Shape Parameter (m)	Log-Likelihood (K)	Number of Parameters	AICc	ΔAICc	Akaike Weight (W)
von Bertalanffy	0.67	-25.30	4	59.64	0.00	0.55
Gompertz	1.2	-25.70	4	60.42	0.79	0.37
Logistic	2	-27.55	4	64.13	4.49	0.06
Classic	0	-28.92	4	66.86	7.23	0.01

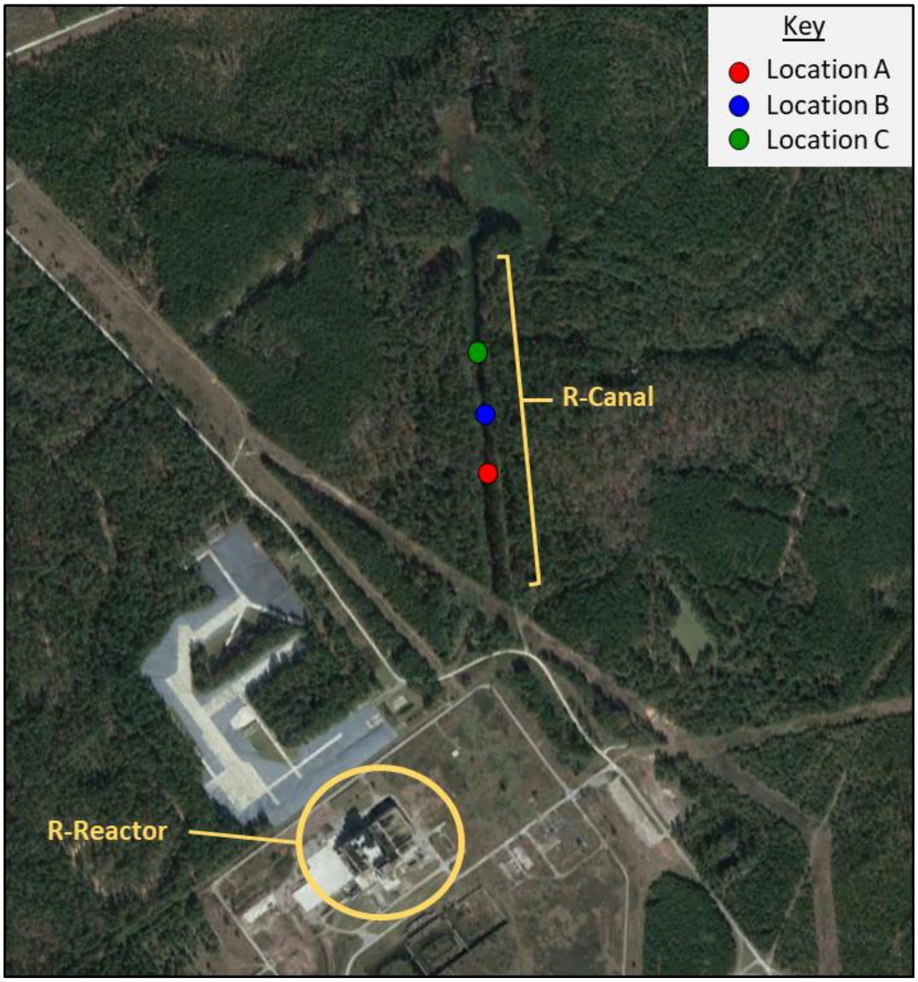

FIGURE 4.1: Locations of experimental enclosures used to contain bullfrog tadpole (*Lithobates catesbianus*) for amphibian radiocesium (^{137}Cs) studies in R-Canal on the U.S. Department of Energy's Savannah River Site in Aiken, SC, USA.

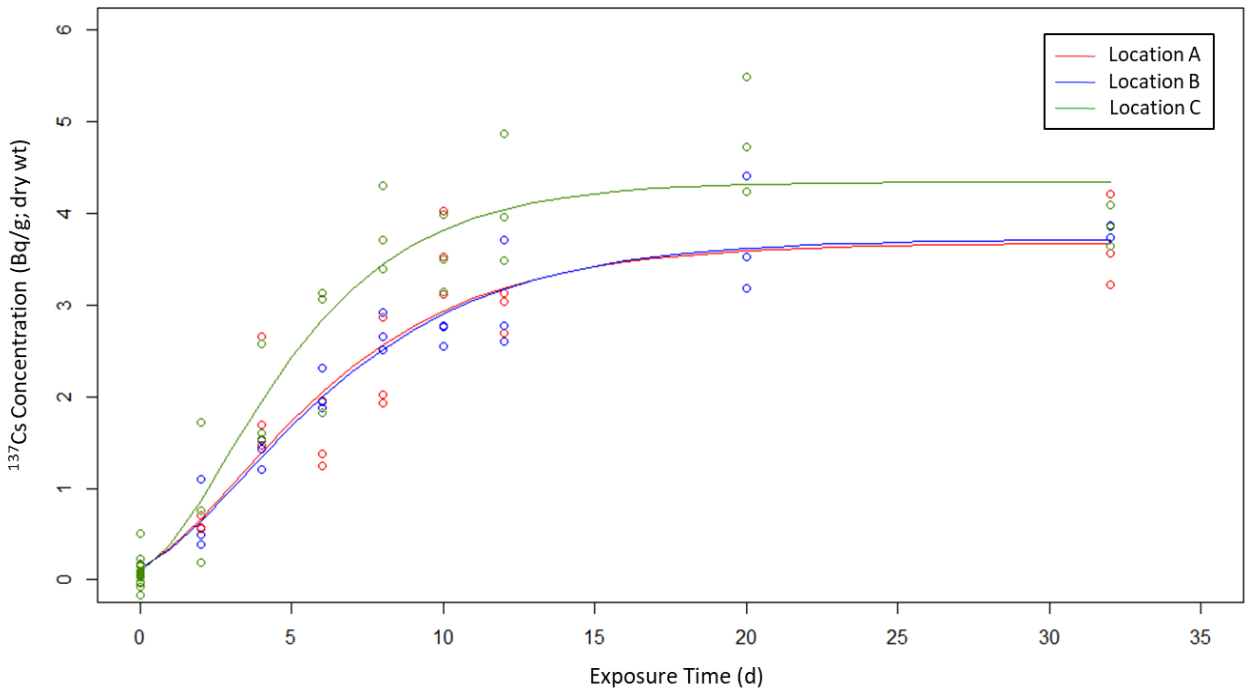

FIGURE 4.2: Accumulation of ^{137}Cs by bullfrog tadpoles (*Lithobates catesbeianus*) restricted in littoral enclosures stationed in three locations within R-Canal of the U.S. Department of Energy's Savannah River Site. Each point represents the collection of a tadpole from one of the three experimental enclosures, except for the points at day = 0, which represent tadpoles analyzed for ^{137}Cs prior to release into the contaminated system. The fitted curve represents the predicted accumulation of ^{137}Cs in tadpoles from each sampling location in R-Canal system using the von Bertalanffy modification of the Richards Model, where the Richards shape parameter m is equal to 0.67.

CHAPTER 5

CONCLUSION

Despite ^{137}Cs contamination spanning the globe as a result of anthropogenic pollution, there are many gaps within the current literature on its fate within the environment as it is incorporated into food webs. To obtain a more holistic understanding of the fate of ^{137}Cs in aquatic and semi-aquatic biota inhabiting contaminated ecosystems, the overarching objectives of my thesis research were to: 1.) elucidate the potential for ^{137}Cs to biomagnify in aquatic food webs that include numerous species of wildlife spanning multiple taxonomic groups, 2.) examine how ^{137}Cs activity contrast between different life stages of frogs and snakes in response to ontogenetic shifts in foraging strategy and diet, and 3.) model ^{137}Cs bioaccumulation in anuran larvae, specifically bullfrogs, to determine the extent of exposure time necessary for tadpoles to reach maximum activity of ^{137}Cs when exposed within contaminated systems. By quantifying ^{137}Cs activity within the tissues of a wide diversity of biota collected from R-Canal on the U.S. Department of Energy's Savannah River Site, the results of my research build on previous studies and provide insight into the dynamics of ^{137}Cs within contaminated ecosystems.

In Chapter 2, I surveyed ^{137}Cs activity across multiple species of aquatic and semi-aquatic wildlife inhabiting a contaminated canal system on the SRS, and examined the potential for ^{137}Cs to biomagnify in food webs where contamination is restricted to the aquatic system. Although biomagnification of ^{137}Cs has been reported in both aquatic and terrestrial ecosystems (Pendleton et al. 1964, Hanson 1967, Carlsson and Lidén 1978, Lowe and Horrill 1991, Rowan et al. 1998, Zhao et al. 2001, Sundbom et al. 2003, Åhman et al. 2004, Pan and Wang 2016), much of this research has been limited to select trophic linkages or taxonomic groups. In contrast, other studies have found higher levels of ^{137}Cs activity in lower trophic levels compared to those at higher trophic positions (Anderson et al. 1973, Straney et al. 1975, Whicker et al. 1990, Rudge et al. 1993, Kålås et al. 1994, Sakai et al. 2016, Ishii et al. 2017), suggesting that ^{137}Cs does not biomagnify in all circumstances. My research supports the latter, as I found that ^{137}Cs concentrations were highly variable among difference species, despite trophic position as determined by stable nitrogen isotopic analysis. This further suggests that ^{137}Cs biomagnification is not ubiquitous, but instead is system dependent and influenced by a suite of biotic and abiotic factors. At my study site, although ^{137}Cs contamination was confined within the effluent canal and primarily located within the sediments within the aquatic system, it was still bioavailable and could be accumulated by plants and animals utilizing the canal. My data revealed that sediment and periphyton samples contained the highest concentrations of ^{137}Cs compared to all invertebrate, fish, amphibian, reptile, and mammal species collected, and ^{137}Cs concentrations were highest in wildlife that heavily consume sediments and periphyton when foraging. I also observed that ^{137}Cs activity was typically lower in semi-aquatic biota compared to those restricted to the aquatic system, likely due to differences in ^{137}Cs exposure through diet and the ability of semi-aquatic organisms to freely move between areas of varying contamination, among other possible biotic and abiotic factors. These factors should always be considered when making any broad assumptions on the ^{137}Cs dynamics in exposed biota inhabiting contaminated aquatic or terrestrial ecosystems.

Expanding on the data collected in Chapter 2, in Chapter 3 I compared ^{137}Cs contamination between different life stages of frogs and snakes in response to ontogenetic shifts in foraging strategy and diet, as well as with increasing body size. Specifically, I compared whole-body ^{137}Cs activity between pre- and post-metamorphosed leopard frogs, green frogs, and bullfrogs, and between juvenile and adult cottonmouths. Unlike other studies in fish and turtles that have shown higher ^{137}Cs concentrations in adult organisms compared to juveniles (Carlsson and Lidén 1978, Rowan et al. 1989, Peters and Brisbin 1996), my data revealed that while cottonmouths did not differ in ^{137}Cs contamination between life stage or with increasing size, tadpoles of each anuran species possessed elevated levels of ^{137}Cs compared to their adult forms. With their propensity to ingest large amounts of contaminated sediments while foraging (Jenssen 1967, Ruibal and Laufer 2012), it is likely that tadpoles are exposed to more ^{137}Cs than post-metamorphic frogs, especially given the larger intestinal surface area relative to body size in tadpoles, allowing for increased contaminant absorption (Hourdry et al. 1996, Roe et al. 2005). I also found that with increasing body size in post-metamorphic bullfrogs, ^{137}Cs activity decreased. This variability among species of different taxonomic groups likely reflects differences in ^{137}Cs exposure through diet and bioavailability within foraging location, as well as underlying differences in behavior and physiology. My results for Chapter 3 demonstrate the need to considering all life stages when evaluating the potential for ^{137}Cs to accumulate in different species of wildlife, as uptake may not be consistent among all life stages within a particular species, or of differing body sizes.

In Chapter 4, I further examined ^{137}Cs accumulation in bullfrog tadpoles to advance our understanding of ^{137}Cs accumulation rates for individuals inhabiting contaminated systems. By constructing large enclosures that were deployed into R-Canal and later stocked with uncontaminated tadpoles, I modeled ^{137}Cs bioaccumulation in bullfrog tadpoles by collecting individuals at known exposure times, and quantified ^{137}Cs activity within their body tissues. Parameter estimates revealed using the Richards Model showed that bullfrog tadpoles reach a maximum, steady-state ^{137}Cs equilibrium after only 14.02 days of exposure. This uptake is relatively rapid compared to bioaccumulation rates in birds (Fendley et al. 1977, Potter 1987, George et al. 1991, Moss and Horrill 1996, Kennamer et al. 2017), turtles (Peters and Brisbin 1996), and fish (Pan and Wang 2016), possibly due to higher exposures through their diet as discussed in previous chapters. These findings further stress the importance of considering amphibians when examining ^{137}Cs concentrations in biota within contaminated ecosystems, and demonstrate the need for examining uptake rates across multiple species of wildlife, as ^{137}Cs bioaccumulation rates can be highly variable among different species and taxonomic groups.

The data presented within my thesis builds upon our current understanding of the fate of ^{137}Cs in biota within contaminated ecosystems. Taking a holistic approach to examining ^{137}Cs levels across a wide range of biota spanning across multiple taxonomic groups, I found that ^{137}Cs concentrations are highly variable despite trophic position, and can presumably be influenced by morphological, behavioral, and physiological characteristics, in addition to diet, life stage, and location within the contaminated system. The conclusions drawn from my master’s research will contribute further information to our understanding of ^{137}Cs dynamics within exposed wildlife, and will contribute towards advancements within the field of ecotoxicology, especially within the scope of radionuclides such as ^{137}Cs.

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